Connecting via Winsock to STN

Welcome to STN International! Enter x:x

LOGINID:ssspta1644pnh

PASSWORD:
TERMINAL (ENTER 1, 2, 3, OR ?):2

* * * * * * * * * * Welcome to STN International * * * * * * * * * *

NEWS	1		Web Page URLs for STN Seminar Schedule - N. America
NEWS	2		"Ask CAS" for self-help around the clock
NEWS	3	FEB 25	CA/CAPLUS - Russian Agency for Patents and Trademarks (ROSPATENT) added to list of core patent offices covered
NEWS	4	FEB 28	PATDPAFULL - New display fields provide for legal status data from INPADOC
NEWS	5	FEB 28	BABS - Current-awareness alerts (SDIs) available
NEWS	6	FEB 28	MEDLINE/LMEDLINE reloaded
NEWS	7	MAR 02	GBFULL: New full-text patent database on STN
NEWS	8	MAR 03	REGISTRY/ZREGISTRY - Sequence annotations enhanced
NEWS	9	MAR 03	MEDLINE file segment of TOXCENTER reloaded
NEWS	10	MAR 22	KOREAPAT now updated monthly; patent information enhanced
NEWS	11	MAR 22	Original IDE display format returns to REGISTRY/ZREGISTRY
NEWS	12	MAR 22	PATDPASPC - New patent database available
NEWS	13	MAR 22	REGISTRY/ZREGISTRY enhanced with experimental property tags
NEWS	14	APR 04	EPFULL enhanced with additional patent information and new fields
NEWS	15	APR 04	EMBASE - Database reloaded and enhanced

NEWS EXPRESS	JANUARY 10 CURRENT WINDOWS VERSION IS V7.01a, CURRENT MACINTOSH VERSION IS V6.0c(ENG) AND V6.0Jc(JP), AND CURRENT DISCOVER FILE IS DATED 10 JANUARY 2005
NEWS HOURS	STN Operating Hours Plus Help Desk Availability
NEWS INTER	General Internet Information
NEWS LOGIN	Welcome Banner and News Items
NEWS PHONE	Direct Dial and Telecommunication Network Access to STN
NEWS WWW	CAS World Wide Web Site (general information)

Enter NEWS followed by the item number or name to see news on that specific topic.

All use of STN is subject to the provisions of the STN Customer agreement. Please note that this agreement limits use to scientific research. Use for software development or design or implementation of commercial gateways or other similar uses is prohibited and may result in loss of user privileges and other penalties.

* * * * * * * * * * * * * * STN Columbus * * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 12:09:11 ON 07 APR 2005

=> file medline embase biosis scisearch caplus

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	0.21	0.21

FILE 'MEDLINE' ENTERED AT 12:09:23 ON 07 APR 2005

FILE 'EMBASE' ENTERED AT 12:09:23 ON 07 APR 2005

FILE 'BIOSIS' ENTERED AT 12:09:23 ON 07 APR 2005

FILE 'SCISEARCH' ENTERED AT 12:09:23 ON 07 APR 2005

FILE 'CAPLUS' ENTERED AT 12:09:23 ON 07 APR 2005

=> s targeting
L1 246964 TARGETING

=> s l1 and intracellular space
L2 86 L1 AND INTRACELLULAR SPACE

=> s l2 and affinity
L3 8 L2 AND AFFINITY

=> dup remove l3
PROCESSING COMPLETED FOR L3
L4 8 DUP REMOVE L3 (0 DUPLICATES REMOVED)

=> d l4 1-8 cbib abs

L4 ANSWER 1 OF 8 EMBASE
on STN
2005089917 EMBASE Locked nucleic acid (LNA) mediated improvements in siRNA stability and functionality. Elmen J.; Thonberg H.; Ljungberg K.; Frieden M.; Westergaard M.; Xu Y.; Wahren B.; Liang Z.; Orum H.; Koch T.; Wahlestedt C.. J. Elmen, Center for Genomics/Bioinformatics, Karolinska Institutet, 171 77 Stockholm, Sweden. joacim.elmen@cgb.ki.se. Nucleic Acids Research Vol. 33, No. 1, pp. 439-447 2005.
Refs: 32.
ISSN: 0305-1048. CODEN: NARHAD
Pub. Country: United Kingdom. Language: English. Summary Language: English.
ED Entered STN: 20050310
AB Therapeutic application of the recently discovered small interfering RNA (siRNA) gene silencing phenomenon will be dependent on improvements in molecule bio-stability, specificity and delivery. To address these issues, we have systematically modified siRNA with the synthetic RNA-like high **affinity** nucleotide analogue, Locked Nucleic Acid (LNA). Here, we show that incorporation of LNA substantially enhances serum half-life of siRNA's, which is a key requirement for therapeutic use. Moreover, we provide evidence that LNA is compatible with the intracellular siRNA machinery and can be used to reduce undesired, sequence-related off-target effects. LNA-modified siRNAs **targeting** the emerging disease SARS, show improved efficiency over unmodified siRNA on certain RNA motifs. The results from this study emphasize LNA's promise in converting siRNA from a functional genomics technology to a therapeutic platform.

L4 ANSWER 2 OF 8 EMBASE
on STN
2005014981 EMBASE Noninvasive imaging of 5-HT(3) receptor trafficking in live cells: From biosynthesis to endocytosis. Ilegems E.; Pickt H.M.; Deluz C.; Kellenberger S.; Vogel H.. H. Vogel, Lab. Phys. Chem. Poly. and Membranes, Swiss Fed. Inst. of Technology-EPFL, CH-1015 Lausanne, Switzerland. horst.vogel@epfl.ch. Journal of Biological Chemistry Vol. 279, No. 51,

pp. 53346-53352 17 Dec 2004.
Refs: 45.
ISSN: 0021-9258. CODEN: JBCHA3
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20050120
AB Sequential stages in the life cycle of the ionotropic 5-HT(3) receptor (5-HT(3)R) were resolved temporally and spatially in live cells by multicolor fluorescence confocal microscopy. The insertion of the enhanced cyan fluorescent protein into the large intracellular loop delivered a fluorescent 5-HT(3)R fully functional in terms of ligand binding specificity and channel activity, which allowed for the first time a complete real-time visualization and documentation of intracellular biogenesis, membrane **targeting**, and ligand-mediated internalization of a receptor belonging to the ligand-gated ion channel superfamily. Fluorescence signals off newly expressed receptors were detectable in the endoplasmic reticulum about 3 h after transfection onset. At this stage receptor subunits assembled to form active ligand binding sites as demonstrated in situ by binding of a fluorescent 5-HT(3)R-specific antagonist. After novel protein synthesis was chemically blocked, the 5-HT(3)R populations in the endoplasmic reticulum and Golgi cisternae moved virtually quantitatively to the cell surface, indicating efficient receptor folding and assembly. Intracellular 5-HT(3) receptors were trafficking in vesicle-like structures along microtubules to the cell surface at a velocity generally below 1 μm/s and were inserted into the plasma membrane in a characteristic cluster distribution overlapping with actin-rich domains. Internalization of cell surface 5-HT (3) receptors was observed within minutes after exposure to an extracellular agonist. Our orchestrated use of spectrally distinguishable fluorescent labels for the receptor, its cognate ligand, and specific organelle markers can be regarded as a general approach allowing subcellular insights into dynamic processes of membrane receptor trafficking.

L4 ANSWER 3 OF 8 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2004172691 EMBASE Tumor cell **targeting** of liposome-entrapped drugs with phospholipid-anchored folic acid-PEG conjugates. Gabizon A.; Shmeeda H.; Horowitz A.T.; Zalipsky S.. A. Gabizon, Oncology Department, Shaare Zedek Medical Center, POB 3235, Jerusalem 91031, Israel. alberto@md.huji.ac.il. Advanced Drug Delivery Reviews Vol. 56, No. 8, pp. 1177-1192 29 Apr 2004.
Refs: 39.
ISSN: 0169-409X. CODEN: ADDREP
S 0169-409X(04)00020-1. Pub. Country: Netherlands. Language: English. Summary Language: English.
ED Entered STN: 20040520
AB **Targeting** of liposomes with phospholipid-anchored folate conjugates is an attractive approach to deliver chemotherapeutic agents to folate receptor (FR) expressing tumors. The use of polyethylene glycol (PEG)-coated liposomes with folate attached to the outer end of a small fraction of phospholipid-anchored PEG molecules appears to be the most appropriate way to combine long-circulating properties critical for liposome deposition in tumors and binding of liposomes to FR on tumor cells. Although a number of important formulation parameters remain to be optimized, there are indications, at least in one ascitic tumor model, that folate **targeting** shifts intra-tumor distribution of liposomes to the cellular compartment. In vitro, folate **targeting** enhances the cytotoxicity of liposomal drugs against FR-expressing tumor cells. In vivo, the therapeutic data are still fragmentary and appear to be formulation- and tumor model-dependent. Further studies are required to determine whether folate **targeting** can confer a clear advantage in efficacy and/or toxicity to liposomal drugs.

L4 ANSWER 4 OF 8 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.

on STN
2004353899 EMBASE Transport of resveratrol, a cancer chemopreventive agent, to cellular targets: Plasmatic protein binding and cell uptake. Jannin B.; Menzel M.; Berlot J.-P.; Delmas D.; Lancon A.; Latruffe N.. N. Latruffe, Lab. de Biol. Molec. et Cell., Universite de Bourgogne, 6 Boulevard Gabriel, 21000, Dijon, France. latruffe@u-bourgogne.fr. Biochemical Pharmacology Vol. 68, No. 6, pp. 1113-1118 15 Sep 2004.
Refs: 32.
ISSN: 0006-2952. CODEN: BCPCA6
S 0006-2952(04)00367-3. Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20040902
AB Resveratrol produced by several plants, berries and fruits, including grapes, is one of the best known natural food microcomponents with potent chemopreventive properties towards the most severe contemporary human diseases: cardiovascular sickness, cancer and neurodegenerative pathologies. Demonstration of its mechanism of action also implies the elucidation of the steps of bioavailability and bioabsorption in cells and tissues. In order to estimate the relationships between the amounts of resveratrol taken up by food or drink intake, and the several possible benefits illustrated from in vitro/in vivo experiments and from epidemiological studies, it is essential to demonstrate step by step the route of resveratrol from plasma to the cell active site. In plasma, resveratrol was shown to interact with lipoproteins. This commentary also contains previously unpublished results about interactions between resveratrol and albumin and the enhancement of this binding in presence of fatty acids. We have previously described that resveratrol uptake by hepatic cells involves two processes - a passive one and a carrier-mediated one. Thanks to this last process, resveratrol, while tightly bound to blood proteins, could be largely delivered to body tissues. The intracellular proteic targets of resveratrol remain to be identified. .COPYRGT. 2004 Elsevier Inc. All rights reserved.

L4 ANSWER 5 OF 8 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
2003488944 EMBASE **Targeting** Stat3 with G-Quartet Oligodeoxynucleotides in Human Cancer Cells. Jing N.; Li Y.; Xu X.; Sha W.; Li P.; Feng L.; Tweardy D.J.. Dr. N. Jing, Section of Infectious Diseases, Department of Medicine, Baylor College of Medicine, Houston, TX 77030, United States. njing@bcm.tmc.edu. DNA and Cell Biology Vol. 22, No. 11, pp. 685-696 2003.
Refs: 39.
ISSN: 1044-5498. CODEN: DCEBE8
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20031229
AB Stat3 is an oncogene that is activated in many human cancer cells. Genetic approaches that disrupt Stat3 activity result in inhibition of cancer cell growth and enhanced cell apoptosis supporting the development of novel drugs **targeting** Stat3 for cancer therapy. G-quartet oligodeoxynucleotides (ODNs) were demonstrated to be potent inhibitors of Stat3 DNA binding activity in vitro with the G-quartet ODN, T40214, having an IC(50) of 7 μM. Computer-simulated docking studies indicated that G-quartet ODNs mainly interacted with the SH2 domain of Stat3 and were capable of inserting between the SH2 domains of Stat3 dimers bound to DNA. We demonstrated that the G-rich ODN T40214, which forms a G-quartet structure at intracellular but not extracellular K+ ion concentrations, is delivered efficiently into the cytoplasm and nucleus of cancer cells where it inhibited IL-6-stimulated Stat3 activation and suppressed Stat3-mediated upregulation of bcl-x and mcl-1 gene expression. Thus, G-quartet represents a new class of drug for **targeting** of Stat3 within cancer cells.

L4 ANSWER 6 OF 8 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
2005101931 EMBASE Adenovirus endocytosis. Meier O.; Greber U.F.. U.F. Greber,

Zoologisches Inst. der Univ. Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland. ufgreber@zool.unizh.ch. Journal of Gene Medicine Vol. 5, No. 6, pp. 451-462 2003.
Refs: 157.
ISSN: 1099-498X. CODEN: JGMEFG
Pub. Country: United Kingdom. Language: English. Summary Language: English.
ED Entered STN: 20050317
AB Pathogen entry into cells occurs by direct penetration of the plasma membrane, clathrin-mediated endocytosis, caveolar endocytosis, pinocytosis or macropinocytosis. For a particular agent, the infectious pathways are typically restricted, reflecting a tight relationship with the host. Here, we survey the uptake process of human adenovirus (Ad) type 2 and 5 and integrate it into the cell biology of endocytosis. Ad2 and Ad5 naturally infect respiratory epithelial cells. They bind to a primary receptor, the coxsackie virus B Ad receptor (CAR). The CAR-docked particles activate integrin coreceptors and this triggers a variety of cell responses, including endocytosis. Ad2/Ad5 endocytosis is clathrin-mediated and involves the large GTPase dynamin and the adaptor protein 2. A second endocytic process is induced simultaneously with viral uptake, macropinocytosis. Together, these pathways are associated with viral infection. Macropinocytosis requires integrins, F-actin, protein kinase C and small G-proteins of the Rho family, but not dynamin. Macropinocytosis per se is not required for vital uptake into epithelial cells, but it appears to be a productive entry pathway of Ad artificially targeted to the high-**affinity** Fcγ receptor CD64 of hematopoietic cells lacking GAR. In epithelial and hematopoietic cells, the macropinosomal contents are released to the cytosol. This requires viral signalling from the surface and coincides with particle escape from endosomes and infection. It emerges that incoming Ad2 and Ad5 distinctly modulate the endocytic trafficking and disrupt selective cellular compartments. These features can be exploited for effective artificial **targeting** of Ad vectors to cell types of interest. Copyright .COPYRGT. 2003 John Wiley & Sons, Ltd.

L4 ANSWER 7 OF 8 MEDLINE on STN
2003506062. PubMed ID: 12864922. A fluorescent cassette-based strategy for engineering multiple domain fusion proteins. Truong Kevin; Khorchid Ahmad; Ikura Mitsuhiko. (Department of Medical Biophysics, University of Toronto, Toronto, M3N 1L6, Canada.. ktruong@uhnres.utoronto.ca) . BMC biotechnology [electronic resource], (2003 Jul 15) 3 (1) 8. Electronic Publication: 2003-07-15. Journal code: 101088663. ISSN: 1472-6750. Pub. country: England: United Kingdom. Language: English.
AB BACKGROUND: The engineering of fusion proteins has become increasingly important and most recently has formed the basis of many biosensors, protein purification systems, and classes of new drugs. Currently, most fusion proteins consist of three or fewer domains, however, more sophisticated designs could easily involve three or more domains. Using traditional subcloning strategies, this requires micromanagement of restriction enzymes sites that results in complex workaround solutions, if any at all. RESULTS: Therefore, to aid in the efficient construction of fusion proteins involving multiple domains, we have created a new expression vector that allows us to rapidly generate a library of cassettes. Cassettes have a standard vector structure based on four specific restriction endonuclease sites and using a subtle property of blunt or compatible cohesive end restriction enzymes, they can be fused in any order and number of times. Furthermore, the insertion of PCR products into our expression vector or the recombination of cassettes can be dramatically simplified by screening for the presence or absence of fluorescence. CONCLUSIONS: Finally, the utility of this new strategy was demonstrated by the creation of basic cassettes for protein **targeting** to subcellular organelles and for protein purification using multiple **affinity** tags.

L4 ANSWER 8 OF 8 EMBASE

on STN
2003218754 EMBASE σ receptors: Potential medications development target for anti-cocaine agents. Matsumoto R.R.; Liu Y.; Lerner M.; Howard E.W.; Brackett D.J.. R.R. Matsumoto, Dept. of Pharmaceutical Sciences, Univ. of OK Health Sciences Center, CPB 337, P.O. Box 26901, Oklahoma City, OK 73190, United States. rae-matsumoto@ouhsc.edu. European Journal of Pharmacology Vol. 469, No. 1-3, pp. 1-12 23 May 2003.
Refs: 124.
ISSN: 0014-2999. CODEN: EJPHAZ
Pub. Country: Netherlands. Language: English. Summary Language: English.
ED Entered STN: 20030612
AB The ability of cocaine to interact with σ receptors suggests a viable target for medications development. Recently, numerous novel compounds and antisense oligodeoxynucleotides **targeting** σ receptors have been synthesized and shown to prevent the behavioral toxicity and psychomotor stimulant effects of cocaine in animals. Protective doses of σ receptor antagonists have also been shown to prevent changes in gene expression that are induced by cocaine. Together, the studies provide insight and promising future directions for the development of potential medications for the treatment of cocaine addiction and overdose. .COPYRGT. 2003 Elsevier Science B.V. All rights reserved.

=> dup remove l2
PROCESSING COMPLETED FOR L2
L5 86 DUP REMOVE L2 (0 DUPLICATES REMOVED)

=> s l5 and FK506 conjugate
L6 0 L5 AND FK506 CONJUGATE

=> s l5 and FK506
L7 0 L5 AND FK506

=> s l1 and FK506
L8 343 L1 AND FK506

=> s l8 and binding affinity
L9 2 L8 AND BINDING AFFINITY

=> dup remove l9
PROCESSING COMPLETED FOR L9
L10 2 DUP REMOVE L9 (0 DUPLICATES REMOVED)

=> d l10 1-2 cbib abs

L10 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN
1999:180895 Document No. 130:346784 Affinity modulation of small-molecule ligands by borrowing endogenous protein surfaces. Briesewitz, Roger; Ray, Gregory T.; Wandless, Thomas J.; Crabtree, Gerald R. (Howard Hughes Medical Institute, Stanford University, Stanford, CA, 94305, USA). Proceedings of the National Academy of Sciences of the United States of America, 96(5), 1953-1958 (English) 1999. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB A general strategy is described for improving the binding properties of small-mol. ligands to protein targets. A bifunctional mol. is created by chemical linking a ligand of interest to another small mol. that binds tightly to a second protein. When the ligand of interest is presented to the target protein by the second protein, addnl. protein-protein interactions outside of the ligand-binding sites serve either to increase or decrease the affinity of the binding event. We have applied this approach to an intractable target, the SH2 domain, and demonstrate a 3-fold enhancement over the natural peptide. This approach provides a way to modulate the potency and specificity of biol. active compds.

L10 ANSWER 2 OF 2 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

92199869 EMBASE Document No.: 1992199869. Inhibition of T cell signaling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. Liu J.; Albers M.W.; Wandless T.J.; Luan S.; Alberg D.G.; Belshaw P.J.; Cohen P.; MacKintosh C.; Klee C.B.; Schreiber S.L.. Department of Chemistry, Harvard University,Cambridge, MA 02138, United States. Biochemistry Vol. 31, No. 16, pp. 3896-3901 1992.
ISSN: 0006-2960. CODEN: BICHAW
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 920802

AB Calcineurin, a Ca2+, calmodulin-dependent protein phosphatase, was recently found to bind with high affinity to two different immunosuppressant binding proteins (immunophilins) with absolute dependence on the presence of the immunosuppressants **FK506** or cyclosporin A (CsA) [Liu et al. (1991) Cell 66, 807-815]. The **binding affinities** of the immunophilin-drug complexes toward calcineurin and the stoichiometry of the resultant multimeric complexes have now been determined, and structural elements of **FK506**, CsA, and calcineurin that are critical for mediating their interactions have been identified. Analogues of **FK506** (FK520, FK523, 15-O-demethyl-FK520) and CsA (MeBm2t1-CsA and MeAla6-CsA) whose affinities for their cognate immunophilins do not correlate with their immunosuppressive activities have been prepared and evaluated in biochemical and cellular assays. We demonstrate a strong correlation between the ability of these analogues, when bound to their immunophilins, to inhibit the phosphatase activity of calcineurin and their ability to inhibit transcriptional activation by NF-AT, a T cell specific transcription factor that regulates IL-2 gene synthesis in human T cells. In addition, FKBP-**FK506** and CyP-CsA do not inhibit members of the PP1, PP2A, and PP2C classes of serine/threonine phosphatases. These data suggest that calcineurin is the relevant cellular target of these immunosuppressive agents and is involved in Ca2+-dependent signal transduction pathways in, among others, T cells and mast cells.

```
=> s FK506 conjugate
L11          12 FK506 CONJUGATE

=> dup remove l11
PROCESSING COMPLETED FOR L11
L12           8 DUP REMOVE L11 (4 DUPLICATES REMOVED)

=> d l12 1-8 cbib abs
```

L12 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

2004:182368 Document No. 140:229401 Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands. Come, Jon H.; Becker, Frank; Kley, Nikolai A.; Reichel, Christoph (USA). U.S. Pat. Appl. Publ. US 2004043388 A1 20040304, 238 pp., Cont.-in-part of U.S. Ser. No. 91,177. (English). CODEN: USXXCO. APPLICATION: US 2002-234985 20020903. PRIORITY: US 2001-PV272932 20010302; US 2001-PV278233 20010323; US 2001-PV329437 20011015; US 2002-91177 20020304.

AB The invention provides compns. and methods for isolating ligand-binding polypeptides for a user-specified ligand, and for isolating small mol. ligands for a user-specified target polypeptide using an improved class of hybrid ligand compds. Preparation of compds., e.g a methotrexate moiety linked by a polyethylene gycol moiety to dexamethasone, is described.

L12 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

2002:31914 Document No. 136:98820 Yeast three-hybrid system for in vivo drug screening and enzyme evolution using chemical inducers of dimerization. Cornish, Virginia W. (USA). U.S. Pat. Appl. Publ. US 2002004202 A1 20020110, 48 pp., Cont.-in-part of U.S. Ser. No. 490,320. (English). CODEN: USXXCO. APPLICATION: US 2001-768479 20010124. PRIORITY: US

2000-490320 20000124.

AB The disclosed invention relates to the evolution of enzymes in vivo, and drug screening in vivo through the use of chemical inducers of protein dimerization. The subject invention provides a compound having the formula: H1--X--B-Y--H2 wherein each of H1 and H2 may be the same or different and capable of binding to a receptor which is the same or different; wherein each of X and Y may be present or absent and if present, each may be the same or different spacer moiety; and wherein B is an enzyme cleavable moiety. This invention also provides a method of screening proteins for the ability to catalyze bond cleavage or bond formation, comprising the steps of: (a) providing a cell that expresses a pair of fusion proteins which upon dimerization change a cellular readout; (b) providing the compound of the invention which dimerizes the pair of fusion proteins, said compound comprising two portions coupled by a bond that is cleavable or formed by the protein to be screened; and (c) screening for the cellular readout, wherein a change the cellular readout indicates catalysis of bond cleavage or bond formation by the protein to be screened. However, it has not heretofore been suggested to use small mol. induced protein dimerization to screen for catalysis in vivo., and specifically, it has not been suggested to use an enzyme cleavable moiety to link two mols. to dimerize proteins. This invention provides proteins de novo with prescribed binding and catalytic properties and permits screening cDNA libraries based on biochem. function. Practically, we believe that powerful screens in combination with existing randomization techniques will make it possible to take an existing protein fold and evolve it into an enzyme with a new function generating useful catalysts for the pharmaceutical and chemical industries. Since the screen is done in vivo and in both prokaryotes and eukaryotes, the methodol. can be applied to functional genomics and drug discovery. A new chemical inducer of dimerization (CID) was recently developed in Professor Cornish's lab, which uses a heterodimer of methotrexate (MTX) and dexamethasone (DEX) which, when placed in the yeast three-hybrid system, reconstitutes transcription of the lacZ gene. The effects of altering the structure of the DEX-MTX CID and the protein chimeras in the three-hybrid assay were investigated. It was observed that all DEX-MTX CIDs, except the DEX-MTX CID with the shortest chemical linker, showed the ability to induce β-galactosidase levels at levels 400% above strains possessing no CID. The DEX-MTX CIDs showed little or no increase in β-galactosidase levels above background levels in strains where dihydrofolate reductase (DHFR) from E. coli was replaced by DHFR from murine. The three-hybrid system did show some directional preference to the way in which the receptors where fused to the DNA binding domain and the activation domain. These studies have led to a better understanding of the factors that are important in activating transcription in the DEX-MTX yeast three-hybrid system.

L12 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

2002:457123 Document No. 138:82854 Drug receptor identification from multiple tissues using cellular-derived mRNA display libraries. McPherson, Michael; Yang, Yingfei; Hammond, Philip W.; Kreider, Brent L. (Phylos, Inc., Lexington, MA, 02421, USA). Chemistry & Biology, 9(6), 691-698 (English) 2002. CODEN: CBOLE2. ISSN: 1074-5521. Publisher: Cell Press.

AB The use of display technologies to identify small mol. receptors from proteome libraries would provide a significant advantage in drug discovery. We have used mRNA display to select, based on affinity, proteins that bind to a drug of interest. A library of mRNA-protein fusion mols. was constructed from human liver, kidney, and bone marrow transcripts and selected using an immobilized FK506-biotin conjugate. Three rounds of selection produced full-length FKBP12 (FK506 binding protein 12 kDa) as the dominant clone. An analogous method was also used to map the minimal drug binding domain within FKBP12. Using this approach, it is anticipated that mRNA display could eventually play a key role in the discovery and characterization of new drug receptor interactions.

L12 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN
2001:91445 Document No. 134:158472 Synthetic transcriptional modulator ligands and their use in gene regulation with chimeric proteins containing DNA-binding domains and ligand-binding domains. Verdine, Gregory L.; Nyanguile, Origene (President and Fellows of Harvard College, USA). U.S. US 6183965 B1 20010206, 38 pp., Cont.-in-part of U.S. Ser. No. 987,912. (English). CODEN: USXXAM. APPLICATION: US 1998-208057 19981209. PRIORITY: US 1997-987912 19971209.

AB Novel synthetic transcriptional modulators having at least one selected ligand linked to at least one transcriptional modulating portion are described. The transcriptional modulators of the present invention can include a ligand linked to a chemical moiety. These transcriptional modulators can be used to selectively control gene expression and to identify components of the transcriptional machinery. Thus, the covalent conjugate (designated L-1) of FK506 and a 29-amino acid peptide of herpes simplex virus VP16 activator domain stimulates transcription in the presence of the chimeric GAL4-FKBP protein, but was unable to stimulate in the absence of GAL4-FKBP and the activation potential was significantly reduced in the presence of added rapamycin or GST-FKBP. Since acyclic peptides having the natural L stereochem. configuration are highly susceptible to proteolysis, the analogous conjugate (D-1) bearing nonnatural D stereochem. is prepared D-1 reproducibly stimulated transcription to a significant extent, though to a slightly lesser extent than L-1. The synthesis of a combinatorial compound library is also provided, and various library components are active transcriptional modulators when coupled to the HATU analog of FK506.

L12 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN
2000:116860 Document No. 132:171073 Conjugates targeted to target receptors and/or interleukin-2 receptors. Prakash, Ramesh K.; Clemens, Christopher M. (Watson Laboratories, Inc., USA). PCT Int. Appl. WO 2000007543 A2 20000217, 67 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US17648 19990804. PRIORITY: US 1998-128572 19980804.

AB A composition for intracellular delivery of a chemical agent into a target receptor and/or interleukin-2-receptor-bearing cell, e.g. an activated T cell and cancer cell, includes a chemical agent, at least one copy of target-receptor binding and/or an interleukin-2-receptor-binding and endocytosis-inducing ligand coupled to a water soluble polymer. The ligand binds to a target receptor and/or IL-2 receptor on the target receptor and/or IL-2-receptor-bearing cell and elicits endocytosis of the composition The composition also optionally includes a biodegradable spacer for coupling the chemical agent and the ligand to the polymer. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. A preferred water soluble polymer is polyalkylene oxide, such as polyethylene glycol and polyethylene oxide, and activated derivs. thereof. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate. Methods of using these compns. for delivering a chemical agent in vivo or in vitro are also disclosed. A method of detecting a disease, such as cancer, T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma, associated with elevated levels of soluble target receptor and/or IL-2 receptor is also disclosed.

L12 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN
1998:787762 Document No. 130:153896 Development of a macromolecular prodrug of FK506: I. Synthesis of FK506-dextran conjugate. Yura, H.; Yoshimura,

N.; Hamashima, T.; Oka, T.; Takakura, Y.; Hashida, M. (Department of Drug Delivery Research, Graduate School of Pharmaceutical Sciences, Kyoto University, Kyoto, Japan). Transplantation Proceedings, 30(7), 3598-3599 (English) 1998. CODEN: TRPPA8. ISSN: 0041-1345. Publisher: Elsevier Science Inc..

AB Activated tacrolimus FK506-hemisuccinate and dextran conjugates were prepared as prodrugs via coupling reactions.

L12 ANSWER 7 OF 8 MEDLINE on STN DUPLICATE 1

97262268. PubMed ID: 9108651. Tacrolimus (FK506): validation of a sensitive enzyme-linked immunosorbent assay kit for and application to a clinical pharmacokinetic study. Lee J W; Sukovaty R L; Farmen R H; Dressler D E; Alak A; Bekersky I. (Harris Laboratories, Lincoln, Nebraska 68501, USA.) Therapeutic drug monitoring, (1997 Apr) 19 (2) 201-7. Journal code: 7909660. ISSN: 0163-4356. Pub. country: United States. Language: English.

AB Tacrolimus (FK506) is a macrolide immunosuppressant approved for the prophylaxis of organ rejection in liver transplant. Immunoassays of low intra- and interday variability and high sensitivity are necessary to adequately characterize terminal elimination phase concentrations in pharmacokinetic studies. A new ELISA kit for the quantitation of tacrolimus in human whole blood has been validated for use in pharmacokinetic studies. Methanol sample extracts were dried and reconstituted in a horseradish peroxidase (HPR)-**FK506 conjugate** solution. The reconstituted samples and mouse anti-FK506 were added to a microplate, precoated with secondary antibody, and incubated, FK506 and the HPR-**FK506 conjugate** competed to bind with anti-FK506, which was immobilized by binding to the secondary antibody. Unbound FK506 was washed away, and substrate was added for color development. Once the reaction was stopped with 2 N H2SO4, the plate was read at 450 nm. The linear range was 0.5-60 ng/ml, with a limit of quantitation of 0.5 ng/ml. Interday precision and accuracy were < or = 10.4% C.V. and < or = 3% R.E. for quality control samples. The lack of interference from endogenous compounds was established by parallelism and recoveries of FK506 from six lots of control matrix. Cross-reactivity against the metabolites and analogs were not performed because the kit monoclonal antibody was from the same source as Kobayashi et al (1). The utility and sensitivity of the kit present a good method for the quantitation of tacrolimus in blood from pharmacokinetic studies. The method is robust and has been used to assay tacrolimus in several thousand whole blood samples by multiple analysts.

L12 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

1994:646278 Document No. 121:246278 Blood cells or blood cell fragments in stable aqueous FK506 standards for FK506 diagnostic analysis. Grenier, Frank C.; Luczkiw, Julie A.; Bergmann, Merry E.; Blonski, David R. (Abbott Laboratories, USA). U.S. US 5338684 A 19940816, 8 pp. Cont. of U.S. Ser. No. 752,410, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1993-71942 19930602. PRIORITY: US 1991-752410 19910830.

AB A stabilized, aqueous composition containing FK506 is disclosed for diagnostic assays for FK506. FK506 degrades rapidly in most aqueous matrixes. The rate of degradation is decreased in the presence of unfixed blood cells or fragments of blood cells from human or animal sources. A number of matrixes using blood components are possible. Blood can be used directly or the blood cells can be lysed. The blood is diluted with a solution of an alkali halide.

```
=> s FK506 and target
L13        2010 FK506 AND TARGET

=> s l13 and FK506 conjugate
L14           2 L13 AND FK506 CONJUGATE

=> dup remove l14
```

PROCESSING COMPLETED FOR L14
L15 2 DUP REMOVE L14 (0 DUPLICATES REMOVED)

=> d l15 1-2 cbib abs

L15 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN
2004:182368 Document No. 140:229401 Three hybrid assay system for isolating ligand-binding polypeptides and for isolating small mol. ligands. Come, Jon H.; Becker, Frank; Kley, Nikolai A.; Reichel, Christoph (USA). U.S. Pat. Appl. Publ. US 2004043388 Â1 20040304, 238 pp., Cont.-in-part of U.S. Ser. No. 91,177. (English). CODEN: USXXCO. APPLICATION: US 2002-234985 20020903. PRIORITY: US 2001-PV272932 20010302; US 2001-PV278233 20010323; US 2001-PV329437 20011015; US 2002-91177 20020304.

AB The invention provides compns. and methods for isolating ligand-binding polypeptides for a user-specified ligand, and for isolating small mol. ligands for a user-specified **target** polypeptide using an improved class of hybrid ligand compds. Preparation of compds., e.g a methotrexate moiety linked by a polyethylene gycol moiety to dexamethasone, is described.

L15 ANSWER 2 OF 2 CAPLUS COPYRIGHT 2005 ACS on STN
2000:116860 Document No. 132:171073 Conjugates targeted to **target** receptors and/or interleukin-2 receptors. Prakash, Ramesh K.; Clemens, Christopher M. (Watson Laboratories, Inc., USA). PCT Int. Appl. WO 2000007543 A2 20000217, 67 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US17648 19990804. PRIORITY: US 1998-128572 19980804.

AB A composition for intracellular delivery of a chemical agent into a **target** receptor and/or interleukin-2-receptor-bearing cell, e.g. an activated T cell and cancer cell, includes a chemical agent, at least one copy of **target**-receptor binding and/or an interleukin-2-receptor-binding and endocytosis-inducing ligand coupled to a water soluble polymer. The ligand binds to a **target** receptor and/or IL-2 receptor on the **target** receptor and/or IL-2-receptor-bearing cell and elicits endocytosis of the composition The composition also optionally includes a biodegradable spacer for coupling the chemical agent and the ligand to the polymer. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. A preferred water soluble polymer is polyalkylene oxide, such as polyethylene glycol and polyethylene oxide, and activated derivs. thereof. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate. Methods of using these compns. for delivering a chemical agent in vivo or in vitro are also disclosed. A method of detecting a disease, such as cancer, T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma, associated with elevated levels of soluble **target** receptor and/or IL-2 receptor is also disclosed.

=> s targeting
L16 246964 TARGETING

=> s l16 and FK506
L17 343 L16 AND FK506

=> s l17 and conjugate
L18 5 L17 AND CONJUGATE

=> dup remove l18

PROCESSING COMPLETED FOR L18
L19 5 DUP REMOVE L18 (0 DUPLICATES REMOVED)

=> d l19 1-5 cbib abs

L19 ANSWER 1 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
2001:380753 Document No. 134:361402 Bifunctional inhibitor molecules, their use in the disruption of protein-protein interactions and therapeutic applications. Crabtree, Gerald R.; Stankunas, Kryn; Briesewitz, Roger; Wandless, Thomas (The Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001036612 A1 20010525, 30 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31695 20001117. PRIORITY: US 1999-PV166675 19991119.

AB Bifunctional inhibitor mols. and methods for their use in the inhibition of protein-protein interactions are provided. The subject bifunctional inhibitor mols. are **conjugates** of a target protein ligand and a blocking protein ligand, where these two moieties are optionally joined by a linking group. In the subject methods, an effective amount of the bifunctional inhibitor mol. is administered to a host in which the inhibition of a protein-protein interaction is desired. The bifunctional inhibitor mol. simultaneously binds to its corresponding target and blocking proteins to produce a tripartite complex that inhibits the target protein-protein interaction. The subject methods and compns. find use in a variety of applications, including therapeutic applications.

L19 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
2001:380414 Document No. 134:371812 Targeted bifunctional molecules and therapies based thereon. Briesewitz, Roger; Crabtree, Gerald R.; Wandless, Thomas (Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001035978 A1 20010525, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31702 20001117. PRIORITY: US 1999-PV166580 19991119.

AB Targeted bifunctional mols. and methods for their use are provided. The subject targeted bifunctional mols. are **conjugates** of a drug moiety and a **targeting** moiety, where these two moieties are optionally joined by a linking group. The bifunctional mols. are further characterized in that they exhibit a modulated biodistribution upon administration to a host as compared to a free drug control. The subject targeted bifunctional mols. find use in a variety of therapeutic applications. For example, a bifunctional mol. consisting of a drug moiety covalently joined to sulfisoxazole which is extensively bound by albumin, via an inert linking group is formed. When this bifunctional mol. enters the human circulation, it is bound by albumin which keeps the drug of interest in the extracellular environment.

L19 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN
2001:91445 Document No. 134:158472 Synthetic transcriptional modulator ligands and their use in gene regulation with chimeric proteins containing DNA-binding domains and ligand-binding domains. Verdine, Gregory L.; Nyanguile, Origene (President and Fellows of Harvard College, USA). U.S. US 6183965 B1 20010206, 38 pp., Cont.-in-part of U.S. Ser. No. 987,912.

(English). CODEN: USXXAM. APPLICATION: US 1998-208057 19981209. PRIORITY: US 1997-987912 19971209.

AB Novel synthetic transcriptional modulators having at least one selected ligand linked to at least one transcriptional modulating portion are described. The transcriptional modulators of the present invention can include a ligand linked to a chemical moiety. These transcriptional modulators can be used to selectively control gene expression and to identify components of the transcriptional machinery. Thus, the covalent **conjugate** (designated L-1) of **FK506** and a 29-amino acid peptide of herpes simplex virus VP16 activator domain stimulates transcription in the presence of the chimeric GAL4-FKBP protein, but was unable to stimulate in the absence of GAL4-FKBP and the activation potential was significantly reduced in the presence of added rapamycin or GST-FKBP. Since acyclic peptides having the natural L stereochem. configuration are highly susceptible to proteolysis, the analogous **conjugate** (D-1) bearing nonnatural D stereochem. is prepared D-1 reproducibly stimulated transcription to a significant extent, though to a slightly lesser extent than L-1. The synthesis of a combinatorial compound library is also provided, and various library components are active transcriptional modulators when coupled to the HATU analog of **FK506.**

L19 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN

2000:116860 Document No. 132:171073 **Conjugates** targeted to target receptors and/or interleukin-2 receptors. Prakash, Ramesh K.; Clemens, Christopher M. (Watson Laboratories, Inc., USA). PCT Int. Appl. WO 2000007543 A2 20000217, 67 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US17648 19990804. PRIORITY: US 1998-128572 19980804.

AB A composition for intracellular delivery of a chemical agent into a target receptor and/or interleukin-2-receptor-bearing cell, e.g. an activated T cell and cancer cell, includes a chemical agent, at least one copy of target-receptor binding and/or an interleukin-2-receptor-binding and endocytosis-inducing ligand coupled to a water soluble polymer. The ligand binds to a target receptor and/or IL-2 receptor on the target receptor and/or IL-2-receptor-bearing cell and elicits endocytosis of the composition The composition also optionally includes a biodegradable spacer for coupling the chemical agent and the ligand to the polymer. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. A preferred water soluble polymer is polyalkylene oxide, such as polyethylene glycol and polyethylene oxide, and activated derivs. thereof. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate. Methods of using these compns. for delivering a chemical agent in vivo or in vitro are also disclosed. A method of detecting a disease, such as cancer, T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma, associated with elevated levels of soluble target receptor and/or IL-2 receptor is also disclosed.

L19 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2005 ACS on STN

1995:881452 Document No. 123:296614 Pretargeting methods and compounds with reduced immunogenicity of **targeting** moiety-anti-ligand **conjugates** or other components employed in diagnostic and therapeutic pretargeting protocols. Graves, Scott S.; Bjorn, Michael J.; Reno, John M.; Axworthy, Donald B.; Fritzberg, Alan R.; Theodore, Louis J. (Neorx Corp., USA). PCT Int. Appl. WO 9515770 A1 19950615, 173 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO

1994-US14223 19941209. PRIORITY: US 1993-164302 19931209.
AB Methods, compds., compns., and kits that relate to pretargeted delivery of diagnostic and therapeutic agents are disclosed. In particular, methods and agents are provided for reducing the immunogenicity of **targeting** moiety-anti-ligand **conjugates** or other components employed in diagnostic and therapeutic pretargeting protocols. Preparation of various **conjugates** for use in the invention is included. Examples include e.g. in vivo anal. of a radiolabeled chelate-biotin **conjugate** administered after antibody pretargeting, clearing agent evaluation, two- and three-step pretargeting methodol., administration of a monoclonal antibody (MAb)-streptavidin **conjugate** in humans, and immunosuppression of MAb-containing **conjugates.**

=> s l16 and rapamycin
L20 885 L16 AND RAPAMYCIN

=> s l20 and conjugate
L21 10 L20 AND CONJUGATE

=> dup remove l21
PROCESSING COMPLETED FOR L21
L22 10 DUP REMOVE L21 (0 DUPLICATES REMOVED)

=> d l22 1-10 cbib abs

L22 ANSWER 1 OF 10 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN
2004517618 EMBASE Conjugation of an antibody to cross-linked fibrin for targeted delivery of anti-restenotic drugs. Thomas A.C.; Campbell J.H.. a.thomas@Bristol.ac.uk. Journal of Controlled Release Vol. 100, No. 3, pp. 357-377 10 Dec 2004.
Refs: 65.
ISSN: 0168-3659. CODEN: JCREEC
S 0168-3659(04)00437-7. Pub. Country: Netherlands. Language: English.
Summary Language: English.
ED Entered STN: 20041217
AB There is an urgent need to treat restenosis, a major complication of the treatment of arteries blocked by atherosclerotic plaque, using local delivery techniques. We observed that cross-linked fibrin (XLF) is deposited at the site of surgical injury of arteries. An antibody to XLF, conjugated to anti-restenotic agents, should deliver the drugs directly and only to the site of injury. An anti-XLF antibody (H93.7C.1D2/48; 1D2) was conjugated to heparin (using N-succinimidyl 3-(2-pyridyldithio)propionate), low molecular weight heparin (LMWH) (adipic acid dihydrazide) and **rapamycin** (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide), and the **conjugates** purified and tested for activity before use in vivo. Rabbits had their right carotid arteries de-endothelialised and then given a bolus of 1D2-heparin, 1D2-LMWH or 1D2-**rapamycin** **conjugate** or controls of saline, heparin, LMWH, **rapamycin** or 1D2 (±heparin bolus) and sacrificed after 2 or 4 weeks (12 groups, n=6/group). Rabbits given any of the **conjugates** had minimal neointimal development in injured arteries, with up to 59% fewer neointimal cells than those given control drugs. Rabbits given 1D2-heparin or 1D2-LMWH had an increased or insignificant reduction in luminal area, with positive remodelling, while the medial and total arterial areas of rabbits given 1D2-**rapamycin** were not affected by injury. Arteries exposed to 1D2-heparin or 1D2-**rapamycin** had more endothelial cells than rabbits given control drugs. Thus, XLF-antibodies can site-deliver anti-restenotic agents to injured areas of the artery wall, where the **conjugates** can influence remodelling, re-endothelialisation and neointimal cell density, with reduced neointimal formation. .COPYRGT. 2004 Elsevier B.V. All rights reserved.

L22 ANSWER 2 OF 10 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2003304172 EMBASE The bullseye of cancer therapy: A moving target. Frost P.; Kamen B.A.. P. Frost, Wyeth Discovery Oncology, Pearl River, NY 10965, United States. Current Opinion in Pharmacology Vol. 3, No. 4, pp. 335-337 2003.
Refs: 9.
ISSN: 1471-4892. CODEN: COPUBK
S 1471-4892(03)00086-9. Pub. Country: United Kingdom. Language: English.
ED Entered STN: 20030814
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L22 ANSWER 3 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2002:754431 Document No. 137:274074 Recombinant production of polyanionic polymers, and uses thereof as drug carriers for improvement of bioactivity and water-solubility. Leung, David W.; Bergman, Philip A.; Lofquist, Alan; Pietz, Gregory E.; Tompkins, Christopher K.; Waggoner, David W., Jr. (Cell Therapeutics Inc, USA). PCT Int. Appl. WO 2002077036 A2 20021003, 74 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2002-US8614 20020321. PRIORITY: US 2001-PV277705 20010321.
AB The invention provides a method for constructing a expression cassette that produce a polyanionic polymer that can be used as drug carriers to improve the bioactivity and water-solubility properties of a drug. The inventive method provides a monodispersed preparation of a recombinantly-produced polyanionic polymer that can be easily manipulated, such as lengthened. An active moiety may be chemical or recombinantly joined to a polyanionic polymer to increase its biol. half-life and/or solubility The instant invention also provides a method for **targeting** the delivery of a polyanionic polymer **conjugate** or fusion protein to a specific cell type or tissue.

L22 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2001:380753 Document No. 134:361402 Bifunctional inhibitor molecules, their use in the disruption of protein-protein interactions and therapeutic applications. Crabtree, Gerald R.; Stankunas, Kryn; Briesewitz, Roger; Wandless, Thomas (The Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001036612 A1 20010525, 30 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31695 20001117. PRIORITY: US 1999-PV166675 19991119.
AB Bifunctional inhibitor mols. and methods for their use in the inhibition of protein-protein interactions are provided. The subject bifunctional inhibitor mols. are **conjugates** of a target protein ligand and a blocking protein ligand, where these two moieties are optionally joined by a linking group. In the subject methods, an effective amount of the bifunctional inhibitor mol. is administered to a host in which the inhibition of a protein-protein interaction is desired. The bifunctional inhibitor mol. simultaneously binds to its corresponding target and blocking proteins to produce a tripartite complex that inhibits the target protein-protein interaction. The subject methods and compns. find use in

a variety of applications, including therapeutic applications.

L22 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2001:380414 Document No. 134:371812 Targeted bifunctional molecules and therapies based thereon. Briesewitz, Roger; Crabtree, Gerald R.; Wandless, Thomas (Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001035978 A1 20010525, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31702 20001117. PRIORITY: US 1999-PV166580 19991119.

AB Targeted bifunctional mols. and methods for their use are provided. The subject targeted bifunctional mols. are **conjugates** of a drug moiety and a **targeting** moiety, where these two moieties are optionally joined by a linking group. The bifunctional mols. are further characterized in that they exhibit a modulated biodistribution upon administration to a host as compared to a free drug control. The subject targeted bifunctional mols. find use in a variety of therapeutic applications. For example, a bifunctional mol. consisting of a drug moiety covalently joined to sulfisoxazole which is extensively bound by albumin, via an inert linking group is formed. When this bifunctional mol. enters the human circulation, it is bound by albumin which keeps the drug of interest in the extracellular environment.

L22 ANSWER 6 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2001:91445 Document No. 134:158472 Synthetic transcriptional modulator ligands and their use in gene regulation with chimeric proteins containing DNA-binding domains and ligand-binding domains. Verdine, Gregory L.; Nyanguile, Origene (President and Fellows of Harvard College, USA). U.S. US 6183965 B1 20010206, 38 pp., Cont.-in-part of U.S. Ser. No. 987,912. (English). CODEN: USXXAM. APPLICATION: US 1998-208057 19981209. PRIORITY: US 1997-987912 19971209.

AB Novel synthetic transcriptional modulators having at least one selected ligand linked to at least one transcriptional modulating portion are described. The transcriptional modulators of the present invention can include a ligand linked to a chemical moiety. These transcriptional modulators can be used to selectively control gene expression and to identify components of the transcriptional machinery. Thus, the covalent **conjugate** (designated L-1) of FK506 and a 29-amino acid peptide of herpes simplex virus VP16 activator domain stimulates transcription in the presence of the chimeric GAL4-FKBP protein, but was unable to stimulate in the absence of GAL4-FKBP and the activation potential was significantly reduced in the presence of added **rapamycin** or GST-FKBP. Since acyclic peptides having the natural L stereochem. configuration are highly susceptible to proteolysis, the analogous **conjugate** (D-1) bearing nonnatural D stereochem. is prepared D-1 reproducibly stimulated transcription to a significant extent, though to a slightly lesser extent than L-1. The synthesis of a combinatorial compound library is also provided, and various library components are active transcriptional modulators when coupled to the HATU analog of FK506.

L22 ANSWER 7 OF 10 CAPLUS COPYRIGHT 2005 ACS on STN
2000:116860 Document No. 132:171073 **Conjugates** targeted to target receptors and/or interleukin-2 receptors. Prakash, Ramesh K.; Clemens, Christopher M. (Watson Laboratories, Inc., USA). PCT Int. Appl. WO 2000007543 A2 20000217, 67 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ,

BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US17648 19990804. PRIORITY: US 1998-128572 19980804.

AB A composition for intracellular delivery of a chemical agent into a target receptor and/or interleukin-2-receptor-bearing cell, e.g. an activated T cell and cancer cell, includes a chemical agent, at least one copy of target-receptor binding and/or an interleukin-2-receptor-binding and endocytosis-inducing ligand coupled to a water soluble polymer. The ligand binds to a target receptor and/or IL-2 receptor on the target receptor and/or IL-2-receptor-bearing cell and elicits endocytosis of the composition The composition also optionally includes a biodegradable spacer for coupling the chemical agent and the ligand to the polymer. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. A preferred water soluble polymer is polyalkylene oxide, such as polyethylene glycol and polyethylene oxide, and activated derivs. thereof. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate. Methods of using these compns. for delivering a chemical agent in vivo or in vitro are also disclosed. A method of detecting a disease, such as cancer, T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma, associated with elevated levels of soluble target receptor and/or IL-2 receptor is also disclosed.

L22 ANSWER 8 OF 10 EMBASE
on STN

1998368440 EMBASE Viral vectors for gene therapy. Robbins P.D.; Ghivizzani S.C.. P.D. Robbins, Department of Molecular Genetics, University of Pittsburgh, W1246 Biomedical Science Tower, Pittsburgh, PA 15261, United States. Pharmacology and Therapeutics Vol. 80, No. 1, pp. 35-47 1998. Refs: 75.
ISSN: 0163-7258. CODEN: PHTHDT
S 0163-7258(98)00020-5. Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 19990122

AB Viruses have evolved to become highly efficient at nucleic acid delivery to specific cell types while avoiding immunosurveillance by an infected host. These properties make viruses attractive gene-delivery vehicles, or vectors, for gene therapy. Several types of viruses, including retrovirus, adenovirus, adeno-associated virus (AAV), and herpes simplex virus, have been modified in the laboratory for use in gene therapy applications. Because these vector systems have unique advantages and limitations, each has applications for which it is best suited. Retroviral vectors can permanently integrate into the genome of the infected cell, but require mitotic cell division for transduction. Adenoviral vectors can efficiently deliver genes to a wide variety of dividing and nondividing cell types, but immune elimination of infected cells often limits gene expression in vivo. Herpes simplex virus can deliver large amounts of exogenous DNA; however, cytotoxicity and maintenance of transgene expression remain as obstacles. AAV also infects many nondividing and dividing cell types, but has a limited DNA capacity. Alternatively, chimeric viral-vector systems that combine advantageous properties of two or more viral systems are also being explored. Although viral-mediated gene delivery has proved to be the most efficient means of gene transfer, nonviral means are also under development. Many of these nonviral systems incorporate portions of viral vectors to increase the efficiency of gene delivery or expression. Retrovirus, adenovirus, and AAV vectors are being evaluated currently in several Phase 1 clinical trials for treatment of diseases such as cancer, cystic fibrosis, Gaucher disease, and arthritis.

L22 ANSWER 9 OF 10 CAPLUS

1995:881452 Document No. 123:296614 Pretargeting methods and compounds with reduced immunogenicity of **targeting** moiety-anti-ligand

conjugates or other components employed in diagnostic and therapeutic pretargeting protocols. Graves, Scott S.; Bjorn, Michael J.; Reno, John M.; Axworthy, Donald B.; Fritzberg, Alan R.; Theodore, Louis J. (Neorx Corp., USA). PCT Int. Appl. WO 9515770 A1 19950615, 173 pp. DESIGNATED STATES: W: CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US14223 19941209. PRIORITY: US 1993-164302 19931209.

AB Methods, compds., compns., and kits that relate to pretargeted delivery of diagnostic and therapeutic agents are disclosed. In particular, methods and agents are provided for reducing the immunogenicity of **targeting** moiety-anti-ligand **conjugates** or other components employed in diagnostic and therapeutic pretargeting protocols. Preparation of various **conjugates** for use in the invention is included. Examples include e.g. in vivo anal. of a radiolabeled chelate-biotin **conjugate** administered after antibody pretargeting, clearing agent evaluation, two- and three-step pretargeting methodol., administration of a monoclonal antibody (MAb)-streptavidin **conjugate** in humans, and immunosuppression of MAb-containing **conjugates.**

L22 ANSWER 10 OF 10 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

93077508 EMBASE Document No.: 1993077508. Transplant rejection: Mechanisms and treatment. Chandler C.; Passaro Jr. E.. Surgical Service, Veterans Affairs Medical Center,Los Angeles, CA 90073, United States. Archives of Surgery Vol. 128, No. 3, pp. 279-283 1993.
ISSN: 0004-0010. CODEN: ARSUAX
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 930418

AB In this review, we summarize the cellular and molecular events in the rejection of transplanted allografts, as well as the rationale for the evolving techniques to suppress such rejection. Allogenic major histocompatibility complex antigens expressed on the allograft and/or on the 'passenger leukocytes' within the graft are the major antigenic stimuli recognized as being foreign by receptors of CD4+/T helper cells of the host. Host macrophages provide a second signal, interleukin (IL) 1, essential to the activation of T helper cells. Subsequent production of IL-2 by T helper cells leads to activation and proliferation of cytotoxic T cells and lymphokine-activated killer cells and the release of IL-4 and IL-6. In addition, IL-2 promotes release of interferon gamma as well as tumor necrosis factor and other proinflammatory cytokines. Therapeutic options to 'downregulate' this cascade have gradually evolved from global nonspecific immunosuppressive techniques (total body irradiation, antilymphocyte serum) to increasingly specific modalities currently being studied, including monoclonal antibodies against the IL-2 receptor (thus **targeting** only vigorously proliferating T cells), antibodies against specific cytokines (interferon gamma, tumor necrosis factor), and now 'designer' antibody-toxin **conjugate** molecules that deliver toxins to selected receptor targets. Finally, work continues toward inducing preoperative antigen-specific (graft) tolerance, including utilization of gene transfection techniques to transfect donor major histocompatibility complex antigens to recipients before surgery, which has been shown to prolong murine cardiac allografts, perhaps by priming specific suppressor cells. Further understanding of the initiation of, and subsequent events in, transplantation rejection will lead to increasingly effective prolongation of graft survival while minimizing adverse effects on the host.

```
=> s l16 and intracellular
L23       21076 L16 AND INTRACELLULAR

=> s l23 and clycosporin A
L24           0 L23 AND CLYCOSPORIN A
```

=> s l23 and FK506
L25 64 L23 AND FK506

=> dup remove l25
PROCESSING COMPLETED FOR L25
L26 32 DUP REMOVE L25 (32 DUPLICATES REMOVED)

=> d l26 1-32 cbib abs

L26 ANSWER 1 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN
2005:331509 The Genuine Article (R) Number: 906PM. Gene therapy targeted at calcium handling as an approach to the treatment of heart failure. Hoshijima M (Reprint). Univ Calif San Diego, Inst Mol Med, Dept Med, Sch Med, UCSD0641, 9500 Gilman Dr, La Jolla, CA 92093 USA (Reprint); Univ Calif San Diego, Inst Mol Med, Dept Med, Sch Med, La Jolla, CA 92093 USA. PHARMACOLOGY & THERAPEUTICS (MAR 2005) Vol. 105, No. 3, pp. 211-228. Publisher: PERGAMON-ELSEVIER SCIENCE LTD. THE BOULEVARD, LANGFORD LANE, KIDLINGTON, OXFORD OX5 1GB, ENGLAND. ISSN: 0163-7258. Pub. country: USA. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS
AB Chronic congestive heart failure primarily of ischemic origin remains a leading cause of morbidity and mortality in the United States and other leading countries. The current main stream of therapy is, however, palliative and uses a complex regimen of drugs, the actions of which are not understood completely. On the other hand, unfavorable remodeling after cardiac injuries of multiple causes has been thought to lead to cardiac contractile dysfunction in heart failure, and a body of scientific evidence points to a central role of intrinsic defects in **intracellular** calcium handling in cardiomyocytes that arise from the distorted functions of several key regulatory molecules on plasma membrane or sarcoplasmic reticulurn (SR), a muscle-specific **intracellular** membrane complex that stores calcium at high concentration. Accordingly, the initial appetite to use gene transfer strategies to modulate calcium regulatory proteins was to validate molecular targets for the development of new pharmaceuticals; however, remarkable therapeutic efficacies found in an initial series of studies using various heart failure animal models immediately promoted us to seek ways to directly apply gene transfer to cure clinical heart failure. The first part of this article reviews our up-to-date knowledge of various functional components to regulate calcium handling in cardiomyocytes, including beta-adrenergic receptor, L-type calcium channel, ryanodine receptor (RyR) and its associated proteins, sarco-endoplasmic reticulum calcium ATPase (SERCA), and phospholamban (PLN), and their abnormalities in failing hearts. A series of new somatic gene transfer attempts **targeting** calcium handling in cardiomyocytes are discussed thereafter. (c) 2004 Elsevier Inc. All rights reserved.

L26 ANSWER 2 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2004375567 EMBASE Calcium-calcineurin signaling in the regulation of cardiac hypertrophy. Wilkins B.J.; Molkentin J.D.. jeff.molkentin@cchmc.org. Biochemical and Biophysical Research Communications Vol. 322, No. 4, pp. 1178-1191 1 Oct 2004.
Refs: 149.
ISSN: 0006-291X. CODEN: BBRCA
S 0006-291X(04)01548-7. Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20040924
AB Cardiac hypertrophy is a leading predicator of progressive heart disease that often leads to heart failure and a loss of cardiac contractile performance associated with profound alterations in **intracellular** calcium handling. Recent investigation has centered on identifying the molecular signaling pathways that regulate cardiac myocyte hypertrophy, as well as the mechanisms whereby alterations in calcium handling are

associated with progressive heart failure. One potential focal regulator of cardiomyocyte hypertrophy that also responds to altered calcium handling is the calmodulin-activated serine/threonine protein phosphatase calcineurin (PP2B). Once activated by increases in calcium, calcineurin mediates the hypertrophic response through its downstream transcriptional effector nuclear factor of activated T cells (NFAT), which is directly dephosphorylated by calcineurin resulting in nuclear translocation. While previous studies have convincingly demonstrated the sufficiency of calcineurin to mediate cardiac hypertrophy and progressive heart failure, its necessity remains an area of ongoing investigation. Here we weigh an increasing body of literature that suggests a causal link between calcineurin signaling and the cardiac hypertrophic response and heart failure through the use of pharmacologic inhibitors (cyclosporine A and **FK506**) and genetic approaches. We will also discuss the manner in which calcineurin-NFAT signaling is negatively regulated in the heart through a diverse array of kinases and inhibitory proteins. Finally, we will discuss emerging theories as to the mechanisms whereby alterations in **intracellular** calcium handling might stimulate calcineurin within the context of a contractile cell continually experiencing calcium flux.

L26 ANSWER 3 OF 32 EMBASE
on STN
2003289134 EMBASE Rapamycin inhibits IL-4-induced dendritic cell maturation in vitro and dendritic cell mobilization and function in vivo. Hackstein H.; Taner T.; Zahorchak A.F.; Morelli A.E.; Logar A.J.; Gessner A.; Thomson A.W.. A.W. Thomson, University of Pittsburgh, Thomas E. Starzl Transplant. Inst., W1544 BST, 200 Lothrop St, Pittsburgh, PA 15213, United States. thomsonaw@msx.upmc.edu. Blood Vol. 101, No. 11, pp. 4457-4463 1 Jun 2003.
Refs: 47.
ISSN: 0006-4971. CODEN: BLOOAW
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20030810
AB Rapamycin (RAPA) is a potent immunosuppressive macrolide hitherto believed to mediate its action primarily via suppression of lymphocyte responses to interleukin 2 (IL-2) and other growth factors. We show here that this view is incomplete and provide evidence that RAPA suppresses the functional activation of dendritic cells (DCs) both in vitro and in vivo. In vitro, RAPA inhibits IL-4-dependent maturation and T-cell stimulatory activity of murine bone marrow-derived DCs. These effects are associated with posttranscriptional down-regulation of both subunits of the IL-4 receptor complex (CD124, CD132) and are mediated via binding of RAPA to its **intracellular** receptor **FK506**-binding protein 12 (FKBP12). In vivo, RAPA impairs steady-state DC generation and fms-like tyrosine 3 kinase ligand (Flt3L) - induced DC mobilization. In addition, in vivo administration of RAPA impairs DC costimulatory molecule up-regulation, production of proinflammatory cytokines, and T-cell allostimulatory capacity. These novel findings have implications for RAPA-based therapy of chronic DC-triggered autoimmune diseases, transplant rejection, and hematologic malignancies with activating Flt3 mutations.

L26 ANSWER 4 OF 32 EMBASE
on STN
2003450522 EMBASE Cyclic ADP-ribose increases Ca(2+) removal in smooth muscle. Bradley K.N.; Currie S.; MacMillan D.; Muir T.C.; McCarron J.G.. J.G. McCarron, Inst. of Biomedical/Life Sciences, Neuroscience and Biomedical Systems, University of Glasgow, West Medical Building, Glasgow G12 8QQ, United Kingdom. j.mccarron@bio.gla.ac.uk. Journal of Cell Science Vol. 116, No. 21, pp. 4291-4306 1 Nov 2003.
Refs: 63.
ISSN: 0021-9533. CODEN: JNCSAI
Pub. Country: United Kingdom. Language: English. Summary Language: English.

ED Entered STN: 20031120

AB Ca(2+) release via ryanodine receptors (RyRs) is vital in cell signalling and regulates diverse activities such as gene expression and excitation-contraction coupling. Cyclic ADP ribose (cADPR), a proposed modulator of RyR activity, releases Ca(2+) from the **intracellular** store in sea urchin eggs but its mechanism of action in other cell types is controversial. In this study, caged cADPR was used to examine the effect of cADPR on Ca(2+) signalling in single voltage-clamped smooth muscle cells that have RyR but lack FKBP12.6, a proposed target for cADPR. Although cADPR released Ca(2+) in sea urchin eggs (a positive control), it failed to alter global or subsarcolemma. [Ca(2+)](c), to cause Ca(2+)-induced Ca(2+) release or to enhance caffeine responses in colonic myocytes. By contrast, caffeine (an accepted modulator of RyR) was effective in these respects. The lack of cADPR activity on Ca(2+) release was unaffected by the introduction of recombinant FKBP12.6 into the myocytes. Indeed in western blots, using brain membrane preparations as a source of FKBP12.6, cADPR did not bind to FKBPs, although **FK506** was effective. However, cADPR increased and its antagonist 8-bromo-cADPR slowed the rate of Ca(2+) removal from the cytoplasm. The evidence indicates that cADPR modulates [Ca(2+)](c) but not via RyR; the mechanism may involve the sarcolemma Ca(2+) pump.

L26 ANSWER 5 OF 32 MEDLINE on STN DUPLICATE 1

2003266324. PubMed ID: 12792519. Superior T-cell suppression by rapamycin and **FK506** over rapamycin and cyclosporine A because of abrogated cytotoxic T-lymphocyte induction, impaired memory responses, and persistent apoptosis. Koenen Hans J P M; Michielsen Etienne C H J; Verstappen Jochem; Fasse Esther; Joosten Irma. (Department for Blood Transfusion and Transplantation Immunology, University Medical Center Nijmegen, The Netherlands.) Transplantation, (2003 May 15) 75 (9) 1581-90. Journal code: 0132144. ISSN: 0041-1337. Pub. country: United States. Language: English.

AB Immunosuppressive therapy is best achieved with a combination of agents **targeting** multiple activation steps of T cells. In transplantation, cyclosporine A (CsA) or tacrolimus (**FK506**) are successfully combined with rapamycin (Rap). Rap and CsA were first considered for combination therapy because **FK506** and Rap target the same **intracellular** protein and thus may act in an antagonistic way. However, in clinical studies, **FK506**+Rap proved to be effective. To date, there is no in vitro data supporting these in vivo findings, and it is unclear whether the observed effects are T-cell mediated. In a human polyclonal allogeneic in vitro model, we found that although combined drug treatment markedly reduced expansion of naive T cells, T-cell activation occurred irrespective of the drug combination used. The induction of cytotoxic effector T cells was reduced by CsA+Rap but completely abolished by **FK506**+Rap. Importantly, combined immunosuppression allowed generation of memory CD4+ and CD8+ T cells and hence did not result in T-cell anergy. However, **FK506** +Rap treatment resulted in a reduced number of allospecific memory T cells showing a decreased cell-cycle turnover and cytokine producing capacity. In contrast, CsA+Rap treatment led to increased memory T-cell numbers responding with elevated kinetics. The ability of Rap to promote apoptosis, which contributes to T-cell suppression, remained unaffected upon combination with **FK506** or CsA. These data support the combined use of **FK506**+Rap over CsA+Rap for immunosuppressive therapy.

L26 ANSWER 6 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2003:403100 The Genuine Article (R) Number: 675PZ. Ca2+-handling proteins and heart failure: Novel molecular targets?. Prestle J (Reprint); Quinn F R; Smith G L. Boehringer Ingelheim KG, Dept Cardiovasc Res, D-88397 Biberach, Germany (Reprint); Univ Glasgow, Inst Biomed & Life Sci, Glasgow, Lanark, Scotland. CURRENT MEDICINAL CHEMISTRY (JUN 2003) Vol. 10, No. 11, pp. 967-981. Publisher: BENTHAM SCIENCE PUBL LTD. PO BOX 1673, 1200 BR

HILVERSUM, NETHERLANDS. ISSN: 0929-8673. Pub. country: Germany; Scotland. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Calcium (Ca2+) ions are the currency of heart muscle activity. During excitation-contraction coupling Ca2+ is rapidly cycled between the cytosol (where it activates the myofilaments) and the sarcoplasmic reticulum (SR), the Ca2+ store. These fluxes occur by the transient activity of Ca2+-pumps and -channels. In the failing human heart, changes in activity and expression profile of Ca2+-handling proteins, in particular the SR Ca2+-ATPase (SERCA2a), are thought to cause an overall reduction in the amount of SR-Ca2+ available for contraction. In the steady state, the Ca2+-content of the SR is essentially a balance between Ca2+-uptake via SERCA2a pump and Ca2+-release via the cardiac SR Ca2+-release channel complex (Ryanodine receptor, RyR2). This review discusses current pharmacological options available to enhance cardiac SR Ca2+ content and the implications of this approach as an inotropic therapy in heart failure. Two options are considered: (i) activation of the SERCA2a pump to increase SR Ca2+-uptake, and (ii) reduction of SR Ca2+-leakage through RyR2. RyR2 forms a macromolecular complex with a number of regulatory proteins that either remain permanently bound or that interact in a time- and/or Ca2+-dependant manner. These regulatory proteins can dramatically affect RyR2 function, e.g. over-expression of the accessory protein FK 506-binding protein 12.6 (FKBP12.6) has recently been shown to reduce SR Ca2+-leak.

Recent attempts to design positive inotropes for chronic administrations have focussed on the use of phosphodiesterase III inhibitors (PDE III inhibitors). These compounds, which increase **intracellular** cAMP-levels, have failed in clinical trials. Therefore medical researchers are seeking new drugs that act through alternative pathways. Novel cardiac inotropes **targeting** SR Ca2+-cycling proteins may have the potential to fill this gap.

L26 ANSWER 7 OF 32 MEDLINE on STN DUPLICATE 2

2003517127. PubMed ID: 12960262. Artificially controlled aggregation of proteins and **targeting** in hematopoietic cells. Rosen Hanna; Gao Ying; Johnsson Ellinor; Olsson Inge. (Department of Hematology, Lund, Sweden.. Hanna.Rosen@hematologi.lu.se) . Journal of leukocyte biology, (2003 Nov) 74 (5) 800-9. Electronic Publication: 2003-07-22. Journal code: 8405628. ISSN: 0741-5400. Pub. country: United States. Language: English.

AB The **targeting** mechanisms for granule proteins in hematopoietic cells are largely unknown. Aggregation is believed to be important for protein sorting-for-entry and sorting-by-retention in endocrine and neuroendocrine cells. We asked whether artificially induced multimerization/aggregation of chimeric proteins could affect their sorting in hematopoietic cells. A system was used that permits ligand-controlled **intracellular** oligomerization of hybrid proteins containing the **FK506**-binding protein (FKBP). The hybrid proteins ELA-(FKBP)3 with neutrophil elastase (ELA) and (FKBP*)4-FCS-hGH with a furin cleavage site (FCS) and human growth hormone (hGH) were expressed in the myeloblastic 32D and the rat basophilic leukemia (RBL-1) hematopoietic cell lines. ELA alone is normally targeted to secretory lysosomes. However, the hybrid proteins and ligand-induced aggregates of them were constitutively secreted and not targeted. The hGH that was released at the FCS in (FKBP*)4-FCS-hGH was also constitutively secreted. We conclude that protein multimerization/aggregation per se is not enough to facilitate sorting-for-entry to secretory lysosomes in hematopoietic cells and that improperly folded proteins may be eliminated from sorting by constitutive secretion.

L26 ANSWER 8 OF 32 MEDLINE on STN DUPLICATE 3

2002627051. PubMed ID: 12384539. Visualization of effective tumor **targeting** by CD8+ natural killer T cells redirected with bispecific antibody F(ab')(2)HER2xCD3. Scheffold Christian; Kornacker Martin; Scheffold Yolanda C; Contag Christopher H; Negrin Robert S.

(Division of Bone Marrow Transplantation, Stanford University School of Medicine, California 94305, USA.) Cancer research, (2002 Oct 15) 62 (20) 5785-91. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB HER2 is an attractive immunotherapeutic target for neoplastic disease because this cell surface molecule is overexpressed on a large fraction of malignant tumor cells. To directly assess therapeutic responses to targeted therapy by noninvasive in vivo imaging in small animals, human HER2-expressing ovarian carcinoma cells were genetically modified with a firefly luciferase gene, and light emission was used for visualization of tumor growth and response to therapy. This imaging approach was able to demonstrate in real-time tumor regression in a HER2 xenograft mouse model by adoptive transfer of in vitro induced and expanded cytotoxic CD8+ natural killer T (NKT) cells retargeted with a humanized bispecific antibody F(ab')(2)HER2xCD3. Immunotherapy with effector cells alone or a humanized monoclonal antibody anti-p185(HER2) (4D5-8) resulted in significant but slower reduction in tumor burden. Long-term survival of tumor xenografts correlated inversely with visible residual tumor burden. In vitro, F(ab')(2)HER2xCD3 substantially augmented cytotoxic activity of CD8+ NKT cells. By flow-sorting, CD8+ NKT cells coexpressing CD56 were found to have the highest redirected killing ability. Treatment with concanamycin A or EGTA abrogated CD8+ NKT cytotoxicity indicating that perforin is a major pathway of tumor cell lysis. In contrast, when CD8+ NKT cell were cross-linked with F(ab')(2)HER2xCD3 neither the immunosuppressants cyclosporine A and **FK506**, nor the increase of **intracellular** cyclic AMP by dibutyryl cyclic AMP were able to inhibit cytotoxicity demonstrating that signaling via the CD3 antigen changes the biological activity of non-MHC-restricted effector cells. These studies have demonstrated that CD8+ NKT cells can be successfully redirected to tumor cells using bispecific antibodies and offer a promising strategy for adoptive immunotherapy of neoplastic diseases.

L26 ANSWER 9 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002150038 EMBASE All of the protein interactions that link steroid receptor.ovrhdot.Hsp90.ovrhdot.immunophilin heterocomplexes to cytoplasmic dynein are common to plant and animal cells. Harrell J.M.; Kurek I.; Breiman A.; Radanyi C.; Renoir J.-M.; Pratt W.B.; Galigniana M.D.. W.B. Pratt, Department of Pharmacology, University of Michigan Med. School, 1301 Med. Sci. Research Building III, Ann Arbor, MI 48109-0632, United States. mgali@umich.edu. Biochemistry Vol. 41, No. 17, pp. 5581-5587 30 Apr 2002.

Refs: 36.

ISSN: 0006-2960. CODEN: BICHAW

Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20020516

AB Both plant and animal cells contain high molecular weight immunophilins that bind via tetratricopeptide repeat (TPR) domains to a TPR acceptor site on the ubiquitous and essential protein chaperone hsp90. These hsp90-binding immunophilins possess the signature peptidylprolyl isomerase (PPIase) domain, but no role for their PPIase activity in protein folding has been demonstrated. From the study of glucocorticoid receptor (GR).ovrhdot.hsp90.ovrhdot.immunophilin complexes in mammalian cells, there is considerable evidence that both hsp90 and the **FK506** -binding immunophilin FKBP52 play a role in receptor movement from the cytoplasm to the nucleus. The role of FKBP52 is to target the GR.ovrhdot.hsp90 complex to the nucleus by binding via its PPIase domain to cytoplasmic dynein, the motor protein responsible for retrograde movement along microtubules. Here, we use rabbit cytoplasmic dynein as a surrogate for the plant homologue to show that two hsp90-binding immunophilins of wheat, wFKBP73 and wFKBP77, bind to dynein. Binding to dynein is blocked by competition with a purified FKBP52 fragment comprising its PPIase domain but is not affected by the immunosuppressant drug **FK506**, suggesting that the PPIase domain but not PPIase activity is involved in dynein binding. The hsp90/hsp70-based chaperone

system of wheat germ lysate assembles complexes between mouse GR and wheat hsp90. These receptor heterocomplexes contain wheat FKBPs, and they bind rabbit cytoplasmic dynein in a PPIase domain-specific manner. Retention by plants of the entire heterocomplex assembly machinery for linking the GR to dynein implies a fundamental role for this process in the biology of the eukaryotic cell.

L26 ANSWER 10 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002313605 EMBASE Identification of novel targets of immunosuppressive agents by cDNA-based microarray analysis. Cristillo A.D.; Bierer B.E.. B.E. Bierer, NHLBI, National Institutes of Health, Bldg. 10, 10 Center Dr., Bethesda, MD 20892, United States. biererb@nih.gov. Journal of Biological Chemistry Vol. 277, No. 6, pp. 4465-4476 8 Feb 2002.
Refs: 76.
ISSN: 0021-9258. CODEN: JBCHA3
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20020926

AB The immunosuppressive agents cyclosporin A (CsA) and tacrolimus (**FK506**) bind to unrelated **intracellular** immunophilin receptors, cyclophilin (CyP) and **FK506**-binding protein (FKBP), respectively. The complexes of CsA.ovrhdot.CyP and of **FK506** .ovrhdot.FKBP both bind to and inhibit the activity of the calcium/calmodulin-dependent serine/threonine phosphatase calcineurin. We used cDNA microarray analysis to characterize early human peripheral blood T cell transcriptional responses following antigen receptor stimulation in the absence or presence of CsA or **FK506**, hoping to identify novel targets dependent upon calcineurin or immunophilins or, perhaps, specific targets of either CyP or FKBP inhibitable by one drug alone. The array data failed to identify genes uniquely sensitive to only one drug, suggesting that transcriptionally regulated, immunophilin-dependent but calcineurin-independent targets fell below the limits of detection in this system. In contrast, transcript profiling identified and mRNA and protein analysis confirmed novel as well as known genes reproducibly induced or inhibited by both immunosuppressive agents. In this context, we show that transcriptional activation of Stat5a and repression of the cytokine interleukin-16 are regulated by T cell receptor engagement and dependent upon drug-immunophilin complexes and, presumably, calcineurin activity.

L26 ANSWER 11 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002269710 EMBASE New compounds for the treatment of eczematous skin diseases. Worm M.. Dr. M. Worm, Universitatsklinikum Charite, Klin. Dermatol. Venerol./Allergol., Schumannstr. 20-21, 10117 Berlin, Germany. margitta.worm@charite.de. Expert Opinion on Therapeutic Patents Vol. 12, No. 7, pp. 1023-1033 2002.
Refs: 52.
ISSN: 1354-3776. CODEN: EOTPEG
Pub. Country: United Kingdom. Language: English. Summary Language: English.

ED Entered STN: 20020815

AB Eczematous skin diseases, like atopic dermatitis and contact dermatitis have been treated with topical glucocorticosteroids for decades. With the introduction of the immunophyllins **FK506** and ascomycin, a new treatment era has begun. The increasing knowledge of pathophysiological interactions and immunological disturbances during the chronic inflammatory process in the skin offers many new therapeutical approaches. These are presented in this review, based on the status of recent patents. Novel therapeutical compounds include cytokine antagonists, cytokine-receptor antagonists, but also molecules interfering with signal transduction pathways. Such molecules inhibit certain **intracellular** signal transducing phosphatases or act at the molecular level of transcription factors. Recent developments target lymphocyte homing through interference of adhesion molecules and chemokine /chemokine receptors. The diversity of these interactions is extensive

and clinical trials will unravel their clinical efficacy. Finally, the developments of glucocorticoid family members, such as retinoids, vitamin D and peroxisome proliferator activated receptor agonists, are discussed. Molecules from members of this family have profound differentiating, antiproliferative, but also immunomodulatory effects, which make them attractive as antieczematous compounds. The design of molecules with high selectivity or in combination formulae highlight them as molecules of interest.

L26 ANSWER 12 OF 32 MEDLINE on STN DUPLICATE 4
2002066220. PubMed ID: 11709498. A differentiation switch for genetically modified hepatocytes. Boccaccio Carla; Ando' Margherita; Comoglio Paolo M. (Institute for Cancer Research and Treatment, University of Torino Medical School, 10060 Candiolo-Torino, Italy.. cboccaccio@ircc.unito.it) . FASEB journal : official publication of the Federation of American Societies for Experimental Biology, (2002 Jan) 16 (1) 120-2. Electronic Publication: 2001-11-14. Journal code: 8804484. ISSN: 1530-6860. Pub. country: United States. Language: English.

AB The hepatocyte growth factor (HGF) receptor mediates a two-sided response-cell proliferation and differentiation. This process, defined as "branching morphogenesis," involves cell scatter and redistribution to form ramified hollow tubules within the extracellular matrix, and protection from apoptosis. We have fused the **intracellular** domain of the HGF receptor (HGFR) with three **FK506**-binding protein (FKBP) domains and a membrane-**targeting** signal. This molecule (FKBP-HGFR) dimerizes after administration of a bifunctional ligand specific for FKBP domains. We show that, in mouse hepatocyte progenitors, FKBP-HGFR dimerization elicits the differentiative side of the HGF response, including cell scatter, morphogenesis, and protection from apoptosis. Surprisingly, FKBP-HGFR does not induce cell proliferation. We could correlate the segregation of the differentiative response with a distinctive signaling kinetic of FKBP-HGFR: a) reduced and prolonged tyrosine kinase activation; and b) low early peak of MAP kinase activation (a log lower than the peak induced by the wild-type receptor), followed by a sustained activation over 6 h. These data show that the biological response triggered by the HGFR can be dissected on the basis of the quantitative signaling profile, and that FKBP-HGFR may be used to control selectively the differentiation of hepatocytes, without promoting cell expansion.

L26 ANSWER 13 OF 32 MEDLINE on STN DUPLICATE 5
2002000539. PubMed ID: 11598113. The conserved sites for the **FK506** -binding proteins in ryanodine receptors and inositol 1,4,5-trisphosphate receptors are structurally and functionally different. Bultynck G; Rossi D; Callewaert G; Missiaen L; Sorrentino V; Parys J B; De Smedt H. (Laboratorium voor Fysiologie, K.U.Leuven Campus Gasthuisberg O/N, Herestraat 49, B-3000 Leuven, Belgium.) Journal of biological chemistry, (2001 Dec 14) 276 (50) 47715-24. Electronic Publication: 2001-10-11. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB We compared the interaction of the **FK506**-binding protein (FKBP) with the type 3 ryanodine receptor (RyR3) and with the type 1 and type 3 inositol 1,4,5-trisphosphate receptor (IP(3)R1 and IP(3)R3), using a quantitative GST-FKBP12 and GST-FKBP12.6 affinity assay. We first characterized and mapped the interaction of the FKBPs with the RyR3. GST-FKBP12 as well as GST-FKBP12.6 were able to bind approximately 30% of the solubilized RyR3. The interaction was completely abolished by **FK506**, strengthened by the addition of Mg(2+), and weakened in the absence of Ca(2+) but was not affected by the addition of cyclic ADP-ribose. By using proteolytic mapping and site-directed mutagenesis, we pinpointed Val(2322), located in the central modulatory domain of the RyR3, as a critical residue for the interaction of RyR3 with FKBPs. Substitution of Val(2322) for leucine (as in IP(3)R1) or isoleucine (as in RyR2) decreased the binding efficiency and shifted the selectivity to FKBP12.6; substitution of Val(2322) for aspartate completely abolished the

FKBP interaction. Importantly, the occurrence of the valylprolyl residue as alpha-helix breaker was an important determinant of FKBP binding. This secondary structure is conserved among the different RyR isoforms but not in the IP(3)R isoforms. A chimeric RyR3/IP(3)R1, containing the core of the FKBP12-binding site of IP(3)R1 in the RyR3 context, retained this secondary structure and was able to interact with FKBPs. In contrast, IP(3)Rs did not interact with the FKBP isoforms. This indicates that the primary sequence in combination with the local structural environment plays an important role in **targeting** the FKBPs to the **intracellular** Ca(2+)-release channels. Structural differences in the FKBP-binding site of RyRs and IP(3)Rs may contribute to the occurrence of a stable interaction between RyR isoforms and FKBPs and to the absence of such interaction with IP(3)Rs.

L26 ANSWER 14 OF 32 MEDLINE on STN DUPLICATE 6

2001341508. PubMed ID: 11278313. Palmitoylation of caveolin-1 in endothelial cells is post-translational but irreversible. Parat M O; Fox P L. (Department of Cell Biology, Cleveland Clinic Foundation, The Lerner Research Institute, Cleveland, Ohio 44195, USA.) Journal of biological chemistry, (2001 May 11) 276 (19) 15776-82. Electronic Publication: 2001-02-13. Journal code: 2985121R. ISSN: 0021-9258. (Investigators: Fox P L, Cleveland Clinic Found, OH) Pub. country: United States. Language: English.

AB Caveolin-1 is a palmitoylated protein involved in assembly of signaling molecules in plasma membrane subdomains termed caveolae and in **intracellular** cholesterol transport. Three cysteine residues in the C terminus of caveolin-1 are subject to palmitoylation, which is not necessary for caveolar **targeting** of caveolin-1. Protein palmitoylation is a post-translational and reversible modification that may be regulated and that in turn may regulate conformation, membrane association, protein-protein interactions, and **intracellular** localization of the target protein. We have undertaken a detailed analysis of [(3)H]palmitate incorporation into caveolin-1 in aortic endothelial cells. The linkage of palmitate to caveolin-1 was hydroxylamine-sensitive and thus presumably a thioester bond. However, contrary to expectations, palmitate incorporation was blocked completely by the protein synthesis inhibitors cycloheximide and puromycin. In parallel experiments to show specificity, palmitoylation of aortic endothelial cell-specific nitric-oxide synthase was unaffected by these reagents. Inhibitors of protein trafficking, brefeldin A and monensin, blocked caveolin-1 palmitoylation, indicating that the modification was not cotranslational but rather required caveolin-1 transport from the endoplasmic reticulum and Golgi to the plasma membrane. In addition, immunophilin chaperones that form complexes with caveolin-1, i.e. **FK506**-binding protein 52, cyclophilin A, and cyclophilin 40, were not necessary for caveolin-1 palmitoylation because agents that bind immunophilins did not inhibit palmitoylation. Pulse-chase experiments showed that caveolin-1 palmitoylation is essentially irreversible because the release of [(3)H]palmitate was not significant even after 24 h. These results show that [(3)H]palmitate incorporation is limited to newly synthesized caveolin-1, not because incorporation only occurs during synthesis but because the continuous presence of palmitate on caveolin-1 prevents subsequent repalmitoylation.

L26 ANSWER 15 OF 32 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:197326 The Genuine Article (R) Number: 405NT. Targeted protein kinase A and PP-2B regulate insulin secretion through reversible phosphorylation. Lester L B (Reprint); Faux M C; Nauert J B; Scott J D. Oregon Hlth Sci Univ, Div Endocrinol, L-607, 3181 SW Sam Jackson Pk Rd, Portland, OR 97201 USA (Reprint); Oregon Hlth Sci Univ, Div Endocrinol, Portland, OR 97201 USA; Oregon Hlth Sci Univ, Portland, OR 97201 USA; Ludwig Inst, Melbourne, Vic, Australia; Howard Hughes Med Inst, Vollum Inst, Portland, OR 97201 USA. ENDOCRINOLOGY (MAR 2001) Vol. 142, No. 3, pp. 1218-1227. Publisher: ENDOCRINE SOC. 4350 EAST WEST HIGHWAY SUITE 500, BETHESDA, MD 20814-4110

USA. ISSN: 0013-7227. Pub. country: USA; Australia. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Protein kinases and phosphatases play key roles in integrating signals from various insulin secretagogues. In this study, we show that the activities of the cAMP-dependent protein kinase (PKA) and the calcium/calmodulin-dependent phosphatase, PP-2B are coordinated resulting in the regulation of insulin secretion. Transient inhibition of PP-2B. using the immunosuppressant **FK506**, increased forskolin stimulated insulin secretion by 2.5-fold +/- 0.3 (n = 6) in rat islets and RINm5F cells. Surprisingly, forskolin treatment resulted in the dephosphorylation of the vesicle-associated protein synapsin 1 and increased PP-2B activity by 2.98 +/- 0.97-fold (n = 4). One potential explanation for the observed coordination of PKA and PP-2B activity is their colocalization through a mutual anchoring protein, AKAP79/150. Accordingly, RINm5F cells expressing AKAP79 exhibited decreased insulin secretion, reduced PP-2B activity and were insensitive to **FK506**. This suggests that AKAP **targeting** of PKA and PP-2B maintains a signal transduction complex that may regulate reversible phosphorylation events involved in insulin secretion.

L26 ANSWER 16 OF 32 EMBASE
on STN

2001079299 EMBASE Human epidermal Langerhans' cells are targets for the immunosuppressive macrolide tacrolimus (**FK506**). Panhans-Gross A.; Novak N.; Kraft S.; Bieber T.. Dr. T. Bieber, Department of Dermatology, Friedrich-Wilhelms-University, Sigmund-Freud-Str 25, D-53105 Bonn, Germany. Journal of Allergy and Clinical Immunology Vol. 107, No. 2, pp. 345-352 2001.
Refs: 46.
ISSN: 0091-6749. CODEN: JACIBY
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20010316

AB Background: The immunosuppressive macrolide tacrolimus (**FK506**) has been shown to inhibit allergic contact dermatitis in animal models as well as in human beings. More recently, successful treatment of atopic dermatitis with an ointment containing tacrolimus has been reported. Objectives: We explored the effects of this compound on epidermal Langerhans' cells (LCs), which are known to play an important pathophysiologic role in inflammatory skin diseases. Methods: The expression of the **intracellular FK506** binding protein (FKBP12) was monitored on freshly isolated and cultured epidermal LCs. Phenotyping and functional exploration of LCs treated with different concentrations of tacrolimus and β-methasone valerate (βMv) were performed. Results: FKBP12 is expressed in freshly isolated LCs but is lost while they are maturating into mature dendritic cells. Tacrolimus inhibited the expression of IL-2R (CD25) and of the costimulatory molecules CD80 (B7.1) and CD40. Expression of MHC class I and II was also affected, whereas CD86 (B7.2) expression was not altered. In contrast, βMv strongly increased the expression of CD25. Paradoxically, while decreasing CD40 and MHC class I expression, βMv significantly increased the expression of MHC class II, CD80, and CD86 on cultured LCs but impaired their allostimulatory activity. Tacrolimus was about 100 times more potent than βMv at inhibiting LC stimulatory function. Conclusion: Tacrolimus can exert immunopharmacologic alterations on LCs, which may account, at least in part, for the therapeutic effect of this compound in eczematous skin diseases.

L26 ANSWER 17 OF 32 SCISEARCH on STN

2001:959297 The Genuine Article (R) Number: 496WR. A differentiation switch for genetically modified hepatocytes. Boccaccio C (Reprint); Ando M; Comoglio P M. IRCC, Str Prov 142, I-10060 Candiolo Torino, Italy (Reprint); Univ Turin, Sch Med, Inst Canc Res & Treatment, I-10060 Candiolo Torino, Italy. FASEB JOURNAL (NOV 2001) Vol. 15, No. 13, pp. U203-U220. Publisher: FEDERATION AMER SOC EXP BIOL. 9650 ROCKVILLE PIKE,

BETHESDA, MD 20814-3998 USA. ISSN: 0892-6638. Pub. country: Italy. Language: English.
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB The hepatocyte growth factor (HGF) receptor mediates a two-sided response-cell proliferation and differentiation. This process, defined as "branching morphogenesis," involves cell scatter and redistribution to form ramified hollow tubules within the extracellular matrix, and protection from apoptosis. We have fused the **intracellular** domain of the HGF receptor (HGFR) with three **FK506**-binding protein (FKBP) domains and a membrane-**targeting** signal. This molecule (FKBP-HGFR) dimerizes after administration of a bifunctional ligand specific for FKBP domains. We show that, in mouse hepatocyte progenitors, FKBP-HGFR dimerization elicits the differentiative side of the HGF response, including cell scatter, morphogenesis, and protection from apoptosis. Surprisingly, FKBP-HGFR does not induce cell proliferation. We could correlate the segregation of the differentiative response with a distinctive signaling kinetic of FKBP-HGFR: a) reduced and prolonged tyrosine kinase activation; and b) low early peak of MAP kinase activation (a log lower than the peak induced by the wild-type receptor), followed by a sustained activation over 6 h. These data show that the biological response triggered by the HGFR can be dissected on the basis of the quantitative signaling profile, and that FKBP-HGFR may be used to control selectively the differentiation of hepatocytes, without promoting cell expansion.

L26 ANSWER 18 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2000:278118 Document No. 132:304288 Regulation of transcription or **intracellular** signaling using fusion proteins containing conditional aggregation domains (CAD). Clackson, Timothy; Rivera, Victor (Ariad Gene Therapeutics, Inc., USA). PCT Int. Appl. WO 2000023600 A1 20000427, 68 pp. DESIGNATED STATES: W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US24328 19991019. PRIORITY: US 1998-PV104752 19981019; US 1998-174767 19981019.

AB A system for regulation of transcription or **intracellular** signaling is developed by using fusion proteins containing conditional aggregation domains (CAD). The fusion proteins containing transcription regulation domain, or DNA binding domain, or cellular localization signal or domain, or cellular signaling domain etc. in addition to one or more CAD (such as FKBP domain or its mutant F36M or W59V) aggregate with one another in the absence of the ligands (rapamycin or its analog AP22542, AP21998, **FK506** etc.), and these aggregated complex disperse following the exposure of the ligands. For transcription regulation, one or two CAD-fusion protein(s) containing a transcription activation or repression domain (TAD or TRD) and/or a DNA binding domain (DB) , is/are introduced to cells with a target gene operably linked to a sequence recognized by DB. For cellular signaling, one or two CAD-fusion protein(s) containing a membrane-**targeting** domain and/or a cellular signaling domain (such as the cytoplasmic domain of a receptor for a growth factor or cytokine), is/are introduced to target cells. These fusion protein are designed to localize at the cell membrane by itself or by being recruited through the other fusion protein to aggregate/disaggregate and induce/block the cellular signal upon the addition of ligands. The system is exemplified by transfecting HT1080 cells with vectors expressing a fusion protein of F36M or W59V FKBP with a DB ZFHD1 and a TAD NF-κB p65 subunit for controlling the switch-on or switch-off of the transcription of the reporter gene driven by ZFHD1 binding site. In addition, the invention provides methods for identifying novel CADs using yeast two hybrid system. This method may be applied for ligand-dependent regulation of many biol. events.

L26 ANSWER 19 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
2000:116860 Document No. 132:171073 Conjugates targeted to target receptors and/or interleukin-2 receptors. Prakash, Ramesh K.; Clemens, Christopher M. (Watson Laboratories, Inc., USA). PCT Int. Appl. WO 2000007543 A2 20000217, 67 pp. DESIGNATED STATES: W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US17648 19990804. PRIORITY: US 1998-128572 19980804.

AB A composition for **intracellular** delivery of a chemical agent into a target receptor and/or interleukin-2-receptor-bearing cell, e.g. an activated T cell and cancer cell, includes a chemical agent, at least one copy of target-receptor binding and/or an interleukin-2-receptor-binding and endocytosis-inducing ligand coupled to a water soluble polymer. The ligand binds to a target receptor and/or IL-2 receptor on the target receptor and/or IL-2-receptor-bearing cell and elicits endocytosis of the composition The composition also optionally includes a biodegradable spacer for coupling the chemical agent and the ligand to the polymer. Chemical agents can include cytotoxins, transforming nucleic acids, gene regulators, labels, antigens, drugs, and the like. A preferred water soluble polymer is polyalkylene oxide, such as polyethylene glycol and polyethylene oxide, and activated derivs. thereof. The composition can further comprise a carrier such as another water soluble polymer, liposome, or particulate. Methods of using these compns. for delivering a chemical agent in vivo or in vitro are also disclosed. A method of detecting a disease, such as cancer, T-cell lymphocytic leukemia, T-cell acute lymphoblastic leukemia, peripheral T-cell lymphoma, Hodgkin's disease, and non-Hodgkin's lymphoma, associated with elevated levels of soluble target receptor and/or IL-2 receptor is also disclosed.

L26 ANSWER 20 OF 32 MEDLINE on STN DUPLICATE 7
2000169496. PubMed ID: 10702636. **FK506**, an immunosuppressant **targeting** calcineurin function. Dumont F J. (Department of Immunology, Merck Research Laboratories, Rahway, NJ 07065, USA.) Current medicinal chemistry, (2000 Jul) 7 (7) 731-48. Ref: 210. Journal code: 9440157. ISSN: 0929-8673. Pub. country: Netherlands. Language: English.

AB The macrolactam natural product, **FK506** (Tacrolimus), acts as a powerful and clinically useful immnosuppressant through disruption of signaling events mediated by the calcium-dependent serine/threonine protein phosphatase, calcineurin (CaN), in T lymphocytes. Its mechanism of action involves the formation of a molecular complex with the **intracellular FK506**-binding protein-12 (FKBP12), thereby acquiring the ability to interact with CaN and to interfere with its access to and dephosphorylation of various substrates. Among the CaN substrates whose activity is altered by **FK506**, the nuclear factors of activated T cells (NFAT), a family of transcription factors regulating lymphokine gene expression, have been shown to play a prominent role in **FK506**-induced immunosuppression. Over the past few years, additional members of the FKBP and NFAT families of proteins have been identified, providing further insights into the complexity of **FK506** biological effects. Furthermore, it has become clear that, predominantly as a result of CaN inhibition, **FK506** alters multiple biochemical processes in a variety of cells besides lymphocytes. This may account for the adverse side effects of the drug, including neurotoxicity and nephrotoxicity. Extensive medicinal chemistry efforts have been devoted to the generation of analogs of **FK506** with the hope of identifying compounds with an improved therapeutic index, that could have broader therapeutic utility than the parent drug. These efforts yielded several compounds with unique biochemical attributes,

showing evidence for a dissociation between immunosuppressive and toxic properties, which may pave the way towards designing safer **FK506** -related immunosuppressants.

L26 ANSWER 21 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
1999:315220 Document No. 131:141025 Cloning, sequencing, and nucleolar **targeting** of the basal-body-binding nucleolar protein BN46/51. Trimbur, Gina M.; Goeckeler, Jennifer L.; Brodsky, Jeffrey L.; Walsh, Charles J. (Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA, 15260, USA). Journal of Cell Science, 112(8), 1159-1168 (English) 1999. CODEN: JNCSAI. ISSN: 0021-9533. Publisher: Company of Biologists Ltd..

AB BN46/51 is an acidic protein found in the granular component of the nucleolus of the amebo-flagellate Naegleria gruberi. When Naegleria amebae differentiate into swimming flagellates, BN46/51 is found associated with the basal body complex at the base of the flagella. In order to determine the factors responsible for **targeting** BN46/51 to a specific subnucleolar region, cDNAs coding for both subunits were isolated and sequenced. Two clones, JG4.1 and JG12.1 representing the 46-kDa and 51-kDa subunits, resp., were investigated in detail. JG12.1 encoded a polypeptide of 263 amino acids with a predicted size of 30.1 kDa that co-migrated with the 51-kDa subunit of BN46/51 when expressed in yeast. JG4.1 encoded a polypeptide of 249 amino acids with a predicted size of 28.8 kDa that co-migrated with the 46-kDa subunit of BN46/51. JG4.1 was identical to JG12.1 except for the addition of an aspartic acid between positions 94 and 95 of the JG12.1 sequence and the absence of 45 amino acids beginning at position 113. The predicted amino acid sequences were not closely related to any previously reported. However, the sequences did have 26-31% identity to a group of FKBPs (**FK506** binding proteins) but lacked the peptidyl-prolyl cis-trans isomerase domain of the FKBPs. Both subunits contained two KKE and three KKX repeats found in other nucleolar proteins and in some microtubule binding proteins. Using "Far Western" blots of nucleolar proteins, BN46/51 bound to polypeptides of 44 kDa and 74 kDa. The 44-kDa component was identified as the Naegleria homolog of fibrillarin. BN46/51 bound specifically to the nucleoli of fixed mammalian cells, cells which lack a BN46/51 related polypeptide. When the JG4.1 and JG12.1 cDNAs were expressed in yeast, each subunit was independently targeted to the yeast nucleolus. We conclude that BN46/51 represents a unique nucleolar protein that can form specific complexes with fibrillarin and other nucleolar proteins. We suggest that the association of BN46/51 with the MTOC of basal bodies may reflect its role in connecting the nucleolus with the MTOC activity for the mitotic spindle. This would provide a mechanism for nucleolar segregation during the closed mitosis of Naegleria amebae.

L26 ANSWER 22 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
1998:734993 Document No. 130:1173 Regulated apoptosis by chimeric proteins binding to **FK506**-type and cyclosporin-type ligands. Crabtree, Gerald R.; Schreiber, Stuart L.; Spencer, David M.; Wandless, Thomas J.; Belshaw, Peter (President & Fellows of Harvard College, USA; Board of Trustees of Leland Stanford Jr. University). U.S. US 5834266 A 19981110, 104 pp., Cont.-in-part of U.S. Ser. No. 179,143, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1994-292597 19940818. PRIORITY: US 1993-17931 19930212; US 1993-92977 19930716; US 1993-93499 19930716; US 1994-179143 19940107; US 1994-179748 19940107.

AB A general procedure is described for the regulated (inducible) dimerization or oligomerization of **intracellular** proteins and methods and materials are presented for using that procedure to regulatably initiate cell-specific apoptosis (programmed cell death) in genetically engineered cells. The procedure involves chimeric (or fused) proteins, DNA constructs encoding them, and ligand mols. capable of oligomerizing the chimeric proteins. The chimeric proteins contain at least one ligand-binding (or receptor) domain fused to an action domain capable of initiating apoptosis within a cell (e.g., Fas or tumor necrosis factor receptor), and may also contain addnl. domains for (1) the

regulatable or constitutive expression of desired genes and (2) **intracellular targeting.** The chimeric proteins are capable of binding to an **FK506**-type ligand, a cyclosporin A-type ligand, tetracycline, or a steroid ligand. One such chimeric protein is myristoylated CD3/FKBP12 (MZF3E) receptor consisting of (1) a c-src fragment sufficient for myristoylation, (2) the cytoplasmic tail of ζ (a component of the B cell receptor), (3) 3 consecutive domains of the FKBP12 immunophilin, and (4) a flu epitope tag; oligomerization/apoptosis is induced by a dimeric derivative of **FK506.** Syntheses are reported for the preparation of dimeric and "bumped" (containing steric bulky groups) derivs. of **FK506** and cyclosporin A. The overall procedures allows ligand-mediated oligomerization for regulated gene therapy.

L26 ANSWER 23 OF 32 EMBASE
on STN
1998237002 EMBASE Targeted expansion of genetically modified bone marrow cells. Jin L.; Siritanaratkul N.; Emery D.W.; Richard R.E.; Kaushansky K.; Papayannopoulou T.; Blau C.A.. C.A. Blau, Div. of Hematology and Med. Genetics, Mailstop 357710, Health Sciences Building, Seattle, WA 98195, United States. tblau@u.washington.edu. Proceedings of the National Academy of Sciences of the United States of America Vol. 95, No. 14, pp. 8093-8097 7 Jul 1998.
Refs: 28.
ISSN: 0027-8424. CODEN: PNASA6
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 19980827
AB The ability to specifically target a mitogenic signal to a population of genetically modified primary cells would have potential applications both for gene and cell therapy. Toward this end, a gene encoding a fusion protein containing the **FK506**-binding protein FKBP12, fused to the **intracellular** portion of the receptor for thrombopoietin (mpl), was introduced into primary murine bone marrow cells. Dimerization of this fusion protein through the addition of a dimeric form of the drug **FK506**, called FK1012, resulted in a marked proliferative expansion of marrow cells that was restricted to the genetically modified population. FK1012's proliferative effect was sustained and reversible. An apparent preference for differentiation along the megakaryocytic lineage was observed. This approach allows for the specific delivery of a mitogenic signal to a population of genetically modified primary cells and may have applications for studies in hematopoiesis and receptor biology, and for gene and cell therapy.

L26 ANSWER 24 OF 32 MEDLINE on STN DUPLICATE 8
97378352. PubMed ID: 9232803. The InsP3 receptor and **intracellular** Ca2+ signaling. Mikoshiba K. (Department of Molecular Neurobiology, Institute of Medical Science University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, 108, Japan.) Current opinion in neurobiology, (1997 Jun) 7 (3) 339-45. Ref: 70. Journal code: 9111376. ISSN: 0959-4388. Pub. country: ENGLAND: United Kingdom. Language: English.
AB The inositol 1,4,5-trisphosphate receptor (InsP3R) is a ligand-gated Ca2+-release channel on **intracellular** Ca2+ store sites (such as the endoplasmic reticulum), and plays an important role in **intracellular** Ca2+ signaling in a wide variety of cell types. Recent studies have shown that binding of inositol 1,4,5-trisphosphate (InsP3) to InsP3R isoforms is differentially regulated by Ca2+, and that InsP3R functions are finely regulated by phosphorylation via tyrosine kinases and protein kinase C, by dephosphorylation via calcineurin, and by binding to FKBP (**FK506**-binding protein). In addition, transient receptor potential (TRP) and TRP-like proteins appear to couple conformationally with the InsP3R for capacitative Ca2+ entry. The importance of InsP3R signaling in neuronal function has been demonstrated by gene **targeting** in mice and by studies of T-cell receptor signaling, apoptosis, meiotic maturation, and cytokinesis.

L26 ANSWER 25 OF 32 EMBASE

on STN DUPLICATE 9
1998132333 EMBASE [Heart transplantation - State of the art today].
HERZTRANSPLANTATION - STATE OF THE ART TODAY. Meiser B.M.; Von Scheidt W.; Weis M.; Bohm D.; Kur F.; Koglin J.; Reichenspurner H.; Uberfuhr P.; Reichart B.. Dr. B.M. Meiser, Herzchirurgische Klinik, Klin. Grosshadern Univ. Munchen, D-81366 Munchen, Germany. Herz Vol. 22, No. 5, pp. 237-252 1997.
Refs: 115.
ISSN: 0340-9937. CODEN: HERZDW
Pub. Country: Germany. Language: German. Summary Language: English; German.
ED Entered STN: 19980602
AB In spite of pharmacological progress, end stage congestive heart failure is still associated with a decrease in quality and expectation of life. Heart transplantation remains the last therapeutic option for these patients. While the one year survival rate has increased over the last few years up to 84%, a major problem remains the significant lack of donors. Therefore, the criteria for the selection of candidates for cardiac transplantation have to be kept quite tight: Evidence of poor outcome without transplantation is associated with ejection fractions below 20 to 25%, cardiac indices less than 2.0 l/min/m2, left ventricular filling pressure above 20 mm Hg and a enddiastolic diameter of > 80 mm. There are, however, also quite important functional parameters indicating the need for heart transplantation, e.g. the maximal oxygene uptake being less than 10 ml/kg/min or below 50% of the age- appropriate value. Elevated pulmonary vascular resistance above 4 to 5 Wood units without a significant decrease during application of prostaglandin derivatives or inhalation of NO represents a contraindication for orthotopic heart transplantation; alternatively, a heterotopic transplantation can be considered. Since there is a significant shortage of suitable donor organs, the donor criteria have been broadened, e.g. the accepted donor age was increased to 60 years. Based on these extended criteria, a careful donor evaluation including cardiac history, cardiac examination, ECG and echocardiogram has to be performed. Coronary angiography in older donors is suggested, but in many cases not possible due to circumstances. Further precondition for a good graft function is a sophisticated donor management until the time of explantation. Hypovolemia and hypocalemia, hypothermia, hypoxia and rapid lost of circulating triiodothyronine (T3) have to be detected and balanced. The cardioplegic solution used might not only have an impact on the immediate postoperative performance of the graft, but also on the long term outcome, particularly with regard to graft vessel disease. There are generally two types of solutions: Those with **intracellular** and those with extracellular electrolyte concentrations. In addition, the potassium concentration might be of some importance. Potassium seems to damage endothelial cells and trigger subsequent immunological reactions. Therefore, high potassium concentrations in the cardioplegic solution might correlate with the incidence of graft vessel disease during the long term follow-up. The surgical technique for orthotopic heart transplantation developed at the beginning of the sixties by Lower and Shumway has been used unchanged for the last 30 years. The only alteration recently introduced is the separate direct anastomosis of the pulmonary and systemic veins in order to improve the atrial function. Until recently the commonly employed immunosuppressive strategy after heart transplantation consisted of the standard drugs cyclosporin, azathioprin and prednisolon. Some transplant-units use additionally induction therapy with antibody preparations. Many centers, however, abolished this regimen due to significant short and long term side effects. Promising new, more specific antibodies (which are chimerized or humanised) could revive the induction concept. The most thoroughly tested novel immunosuppressive agent is tacrolimus (**FK506**). It has been demonstrated to be 10 to 100 times more potent than cyclosporin A in vitro and in vivo models. It binds to a different binding protein (FK- binding-protein) than cyclosporin (cyclophilin), but has a similar mechanism of action inhibiting the expression of T-cell activator genes for certain cytokines. First

non-randomised studies after heart transplantation performed at the University of Pittsburgh revealed that significantly more tacrolimus than cyclosporin patients were free of rejection. In order to confirm these observations, we performed a prospective randomised controlled clinical study. When compared to cyclosporine, tacrolimus proved to be a save and effective immunosuppressant with similar survival rates and a significant reduction in the incidence of acute myocardial rejection. The safety profiles of tacrolimus and cyclosporine appeared to be comparable in most major categories. Furthermore, we found that initial intravenous tacrolimus therapy is highly recommended in the immediate post transplant period. Another new immunosuppressive agent, mycophenolate mofetil (MMF), a morpholinoethyl ester of mycophenolic acid, inhibits the de novo synthesis of purines and acts thus as selective suppressor of the proliferation of both T- and B-lymphocytes. Based on early clinical results from kidney transplantation, a multicenter trial in heart transplantation was designed comparing MMF and azathioprin (in combination with cyclosporine and corticosteroids) after heart transplantation. The study indicated that patients treated with MMF have favourable survival rates. In the meantime, we combined MMF and tacrolimus and found that the efficacy of MMF (in this combination) is highly dependent on MMF-trough levels. Therefore, we are currently adjusting the MMF dosage, **targeting** blood levels of 2.5 to 4 µg/ml. To date, using this regimen, we have been able to prevent rejection after heart transplantation completely. The (with regard to clinical trials) least tested major new in immunosuppressive substances is sirolimus (rapamycin). The molecule is related to tacrolimus and binds to the same protein, but acts later in the activation cascade (G1 phase of the cell cycle). So far, sirolimus has only been used for control of rejection episodes after heart transplantation. It remains to be seen if the substance is also a potent drug for primary immunosuppression after heart transplantation. Any increase of immunosuppression might also be associated with a higher risk for infections. Particularly the cytomegalovirus (CMV) continues to be an important cause of infection and disease in organ transplant recipients. Therefore, improved CMV-prophylaxis using a newly available oral formulation of gangciclovir might reduce or at least delay the onset of CMV infection. Acute rejection reactions and infections represent the most important factors for the outcome only within the first year after transplantation. In the late follow-up, however, graft vessel disease becomes the major problem in cardiac recipients. It is manifested by a unique and unusually accelerated form of coronary disease affecting both intramyocardial and epicardial coronary arteries and veins. The exact pathogenesis remains to be established. While it seems to be a primarily immune-mediated disease, the immunologic mechanisms might be accelerated in a milieu of non-immunologic risk factors like damaged endothelium (caused by ischemia and reperfusion damage), post transplant hypercholesterinemia, hypertension and cytomegalovirus infection. Basically three different morphological types can be differentiated: Early after transplantation, diffuse intimal thickening or vasculitis predominate; late after transplantation, focal artheriosclerotic plaques are found; and in some patients a type of dilative angiopathy occurs. Most non-invasive screening tests are not sensitive or specific enough; only stress- echocardiography seems to be quite reliable, at least for a selective patient cohort. Coronary angiography, however, represents still the standard examination which is yearly performed in these patients. But angiography has also been shown to underestimate the presence of the disease and is certainly not sensitive enough to detect it in its early stage. More recently, intracoronary ultrasound has been introduced as an imaging modality to prove and quantify intimal thickening already in the early postoperative period. Furthermore, intracoronary doppler flow velocity measurement is used to assess the endothelial dependent and independent resistance bed of the coronary microcirculation. Efforts to prevent or treat this disease include an improved immunosuppressive regimen, the application of antiproliferative agents as well as the treatment of accompanying risk factors (e.g. by HMG- CoA reductase inhibitors, calcium channel blockers, ganciclovir). To date, preexisting

graft vessel disease can not be reversed by any known treatment strategies. Since the stenoses are diffuse in most cases, coronary interventions like PTCA or coronary artery bypass graft surgery can only be performed in selective patients as palliative therapy. The last option in patients with endstage vessel disease is retransplantation, which is, however, associated with increased mortality (in comparison to primary transplantation). The clinical diagnosis of graft vessel disease is rather difficult caused by the fact, that the heart graft is denervated and therefore no angina pain is felt. Denervation furthermore results in a lack of neural modulation of heart rate as well as inotropism and therefore leads to a decrease of maximal rate, cardiac output and oxygen uptake. In the long term follow-up, a sympathetic reinnervation can occur. In spite of denervation and graft vessel disease, many transplanted hearts reveal no signs of decrease in long term function. The quality of life of these patients is comparable to that of the general population.

L26 ANSWER 26 OF 32 EMBASE
on STN
97005382 EMBASE Document No.: 1997005382. The immunosuppressant **FK506** and its nonimmunosuppressive analog L- 685,818 are toxic to Cryptococcus neoformans by inhibition of a common target protein. Odom A.; Del Poeta M.; Perfect J.; Heitman J.. J. Heitman, 322 CARL Bldg., Duke University Medical Center, Box 3546, Research Dr., Durham, NC 27710, United States. Antimicrobial Agents and Chemotherapy Vol. 41, No. 1, pp. 156-161 1997. Refs: 52.
ISSN: 0066-4804. CODEN: AMACCQ
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 970128
AB The immunosuppressant **FK506** (tacrolimus) is an antifungal natural product macrolide that suppresses the immune system by blocking T-cell activation. In complex with the **intracellular** protein FKBP12, **FK506** inhibits calcineurin, a Ca2+-calmodulin-dependent serine-threonine protein phosphatase. We recently reported that growth of the opportunistic fungal pathogen Cryptococcus neoformans is resistant to **FK506** at 24°C but sensitive at 37°C and that calcineurin, the target of FKBP12-FKS06, is required for growth at 37°C in vitro and pathogenicity in vivo. These findings identify calcineurin as a potential antifungal drug target. In previous studies the calcineurin inhibitor cyclosporin A (CsA) was effective against murine pulmonary infections but exacerbated cryptococcal meningitis in rabbits and mice, likely because CsA does not cross the blood-brain barrier. Although we find that **FK506** penetrates the CNS, **FK506** also exacerbates cryptococcal meningitis in rabbits. Thus, **FK506** immunosuppression outweighs antifungal action in vivo. Like **FK506**, the nonimmunosuppressive **FK506** analog L-685,818 is toxic to C. neoformans in vitro at 37°C but not at 24°C, and **FK506**- resistant mutants are resistant to L-685,818, indicating a similar mechanism of action. Fluconazole-resistant C. neoformans clinical isolates were also found to be susceptible to both **FK506** and L-685,818. Our findings identify calcineurin as a novel antifungal drug target and suggest the nonimmunosuppressive **FK506** analog L-685,818 or other congeners warrant further consideration as antifungal drugs for C. neoformans.

L26 ANSWER 27 OF 32 CAPLUS
1996:350220 Document No. 125:27701 Regulatable elimination of gene expression, gene product function and engineered host cells, and its application in gene therapy. Brugge, Joan S.; Crabtree, Gerald R. (Ariad Gene Therapeutics, Inc., USA). PCT Int. Appl. WO 9606111 A1 19960229, 141 pp. DESIGNATED STATES: W: AU, CA, GB, JP, KR, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US10591 19950818. PRIORITY: US 1994-292595 19940818; US 1994-292596 19940818; US 1994-292597 19940818.
AB Materials and methods are disclosed for regulated obstruction of the

expression of a target gene or the biol. effect of its gene product in genetically engineered cells or organisms containing them. Aspects of the invention are exemplified by recombinant modifications of host cells and their use in vitro and in vivo for the regulatable blockade of expression of a target gene, for interference with the function or effect of a target gene product or for the regulatable elimination of a target gene. Synthesis of oligomer of ligands such as **FK506** and cyclosporin A, and regulation of programmed cell death with immunophilin-Fas antigen chimeras were demonstrated.

L26 ANSWER 28 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
1995:541403 Document No. 122:283855 Regulated apoptosis by chimeric proteins binding to **FK506**-type and cyclosporin-type ligands. Crabtree, Gerald R.; Schreiber, Stuart L.; Spencer, David M.; Wandless, Thomas J.; Belshaw, Peter (Board of Trustees of the Leland Stanford Junior University, USA; President and Fellows of Harvard College). PCT Int. Appl. WO 9502684 A1 19950126, 134 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US8008 19940718. PRIORITY: US 1993-93499 19930716; US 1994-179143 19940107; WO 1994-US1617 19940214.

AB A general procedure is described for the regulated (inducible) dimerization or oligomerization of **intracellular** proteins and methods and materials are presented for using that procedure to regulatably initiate cell-specific apoptosis (programmed cell death) in genetically engineered cells. The procedure involves chimeric (or fused) proteins, DNA constructs encoding them, and ligand mols. capable of oligomerizing the chimeric proteins. The chimeric proteins contain at least one ligand-binding (or receptor) domain fused to an action domain capable of initiating apoptosis within a cell, and may also contain addnl. domains for (1) the regulatable or constitutive expression of desired genes and (2) **intracellular targeting.** The chimeric proteins are capable of binding to an **FK506**-type ligand, a cyclosporin A-type ligand, tetracycline, or a steroid ligand. One such chimeric protein is myristoylated CD3/FKBP12 (MZF3E) receptor consisting of (1) a c-src fragment sufficient for myristoylation, (2) the cytoplasmic tail of ζ (a component of the B cell receptor), (3) 3 consecutive domains of the FKBP12 immunophilin, and (4) a flu epitope tag; oligomerization/apoptosis is induced by a dimeric derivative of **FK506** . Syntheses are reported for the preparation of dimeric and "bumped" (containing steric bulky groups) derivs. of **FK506** and cyclosporin A. The overall procedures allows ligand-mediated oligomerization for regulated gene therapy.

L26 ANSWER 29 OF 32 CAPLUS COPYRIGHT 2005 ACS on STN
1995:377088 Document No. 122:153359 Regulated transcription of target genes with dimeric ligands which cause chimeric receptor proteins to oligomerize and induce gene transcription. Crabtree, Gerald R.; Schreiber, Stuart L.; Spencer, David M.; Wandless, Thomas J.; Belshaw, Peter (Leland Stanford Junior University, USA; Harvard College). PCT Int. Appl. WO 9418317 A1 19940818, 133 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, SK; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US1617 19940214. PRIORITY: US 1993-17931 19930212; US 1993-92977 19930716; US 1994-179748 19940107.

AB A general procedure for regulating (inducing) dimerization or oligomerization of chimeric proteins is presented. The chimeric proteins contain a receptor domain and another protein domain capable of initiating a biol. process. The chimeric proteins can be induced to associate by treating the cells or organisms that harbor them with cell-permeable,

synthetic ligands. The dimers/oligomers bind to a transcription control element and stimulate transcription of the gene to which it is associated The syntheses of FK-506 dimers are presented. Such dimers were used to induce: (1) the **intracellular** aggregation of the cytoplasmic tail of the zeta chain of the T cell receptor (TCR)-CD3 complex thereby leading to signaling and transcription of a reporter gene, (2) the homodimerization of the cytoplasmic tail of the Fas receptor thereby leading to cell-specific apoptosis (programmed cell death) and (3) the heterodimerization of a DNA-binding domain (Gal4) and a transcription-activation domain (VP16) thereby leading to direct transcription of a reporter gene.

L26 ANSWER 30 OF 32 MEDLINE on STN DUPLICATE 10

94339699. PubMed ID: 8061522. pCyP B: a chloroplast-localized, heat shock-responsive cyclophilin from fava bean. Luan S; Lane W S; Schreiber S L. (Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138.) Plant cell, (1994 Jun) 6 (6) 885-92. Journal code: 9208688. ISSN: 1040-4651. Pub. country: United States. Language: English.

AB When the immunosuppressants cyclosporin A (CsA) and **FK506** bind to their **intracellular** receptors (immunophillins), they form complexes that bind to calcineurin and block calcineurin-dependent signaling pathways in immune cells. Previously, we reported that higher plants also express immunophilins and have a Ca(2+)-dependent signaling pathway sensitive to immunophilin-ligand complexes. Based on an N-terminal peptide sequence of a chloroplast-localized cyclophilin (pCyP B), we isolated a cDNA clone encoding the preprotein of the cyclophilin. The deduced amino acid sequence of this cDNA starts with a putative transit sequence for chloroplast **targeting.** The mature pCyP B protein has rotamase activity with low-substrate specificity. Enzyme activity was inhibited by CsA with an inhibition constant of 3.9 nM. Similar to other CyPs from mammalian cells, pCyP B, when complexed with CsA, inhibited the phosphatase activity of bovine calcineurin. The mRNA level of pCyP B was high in leaf tissue but was not detectable in roots. Expression of the transcript in the leaf tissues was regulated by light and induced by heat shock. These findings illustrate the conserved nature of cyclophilin proteins among all of the eukaryotes and suggest that cyclophilins have a unique mode of regulation in higher plants.

L26 ANSWER 31 OF 32 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

94215832 EMBASE Document No.: 1994215832. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. Brown E.J.; Albers M.W.; Tae Bum Shin; Ichikawa K.; Keith C.T.; Lane W.S.; Schreiber S.L.. Department of Chemistry, Howard Hughes Medical Institute, 12 Oxford Street,Cambridge, MA 02138, United States. Nature Vol. 369, No. 6483, pp. 756-758 1994. ISSN: 0028-0836. CODEN: NATUAS
Pub. Country: United Kingdom. Language: English. Summary Language: English.

ED Entered STN: 940817

AB The structurally related natural products rapamycin and **FK506** bind to the same **intracellular** receptor, FKBP12, yet the resulting complexes interfere with distinct signalling pathways. FKBP12-rapamycin inhibits progression through the G1 phase of the cell cycle in osteosarcoma, liver and T cells as well as in yeast, and interferes with mitogenic signalling pathways that are involved in G1 progression, namely with activation of the protein p70(S6k) (refs 5, 11-13) and cyclin-dependent kinases. Here we isolate a mammalian FKBP-rapamycin-associated protein (FRAP) whose binding to structural variants of rapamycin complexed to FKBP12 correlates with the ability of these ligands to inhibit cell-cycle progression. Peptide sequences from purified bovine FRAP were used to isolate a human cDNA clone that is highly related to the DRR1/TOR1 and DRR2/TOR2 gene products from Saccharomyces cerevisiae. Although it has not been previously demonstrated that either of the DRR/TOR gene products can bind the FKBP-rapamycin complex directly, these yeast genes have been genetically

linked to a rapamycin-sensitive pathway and are thought to encode lipid kinases.

L26 ANSWER 32 OF 32 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

1993:340681 Document No.: PREV199396037681. Structure-function analysis of the Bcl-1 oncoprotein: Addition of a heterologous transmembrane domain to portions of the Bcl-2-beta protein restores function as a regulator of cell survival. Tanaka, Shigeki; Saito, Koichi; Reed, John C. [Reprint author]. La Jolla Cancer Res. Foundation, 10901 N. Torrey Pines Rd., La Jolla, CA 92037, USA. Journal of Biological Chemistry, (1993) Vol. 268, No. 15, pp. 10920-10926.
CODEN: JBCHA3. ISSN: 0021-9258. Language: English.

AB The bcl-2 gene can potentially encode 26-and 22-kDa proteins that differ only in their carboxyl tails because of an alternative splicing mechanism. The larger of these proteins contains a hydrophobic transmembrane domain within its carboxyl terminus, resides (at least in part) in mitochondrial membranes and has been shown to prolong cell survival by blocking programmed cell death (also termed "apoptosis"). To explore the function of the shorter 22-kDa Bcl-2 protein that lacks a transmembrane domain, DNAs encoding p26-Bcl-2-alpha or p22-Bcl-2-beta were expressed in an interleukin-3 (IL-3)-dependent hematopoietic cell line 32D. In contrast to p26-Bcl-2-alpha that markedly prolonged cell survival, p22-Bcl-2-beta did not extend the survival of 32D cells when cultured in the absence of IL-3. Expression in 32D cells of a chimeric DNA that fused portions of the open reading frame common to Bcl-2-alpha and Bcl-2-beta (amino-acids 1-195) with sequences encoding the transmembrane and cytosolic domains of the IL-2 receptor-alpha protein resulted in production of a Bcl-2/IL-2R fusion protein that was capable of prolonging 32D cell survival in the setting of IL-3 withdrawal. Based on fractionation of cells to produce crude heavy membrane, light membrane, nuclei, and cytosolic preparations, much of the p22-Bcl-2-beta protein appeared to reside in the cytosol, whereas Bcl-2-alpha and the Bcl-2/IL-2R chimeric proteins were found exclusively in fractions that also contained the inner mitochondrial membrane protein F-1-beta-ATPase. Taken together, these findings demonstrate the importance of membrane association for the function and **intracellular targeting** of the apoptosis-blocking Bcl-2 protein. Furthermore, despite the strong evolutionary conservation of the carboxyl regions of Bcl-2-alpha proteins observed previously for mammalian and avian species, these data suggest that a heterologous transmembrane domain can be substituted without loss of function.

=> d his

(FILE 'HOME' ENTERED AT 12:09:11 ON 07 APR 2005)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 12:09:23 ON 07 APR 2005

```
L1      246964 S TARGETING
L2          86 S L1 AND INTRACELLULAR SPACE
L3           8 S L2 AND AFFINITY
L4           8 DUP REMOVE L3 (0 DUPLICATES REMOVED)
L5          86 DUP REMOVE L2 (0 DUPLICATES REMOVED)
L6           0 S L5 AND FK506 CONJUGATE
L7           0 S L5 AND FK506
L8         343 S L1 AND FK506
L9           2 S L8 AND BINDING AFFINITY
L10          2 DUP REMOVE L9 (0 DUPLICATES REMOVED)
L11         12 S FK506 CONJUGATE
L12          8 DUP REMOVE L11 (4 DUPLICATES REMOVED)
L13       2010 S FK506 AND TARGET
L14          2 S L13 AND FK506 CONJUGATE
L15          2 DUP REMOVE L14 (0 DUPLICATES REMOVED)
L16     246964 S TARGETING
```

```
L17          343 S L16 AND FK506
L18            5 S L17 AND CONJUGATE
L19            5 DUP REMOVE L18 (0 DUPLICATES REMOVED)
L20          885 S L16 AND RAPAMYCIN
L21           10 S L20 AND CONJUGATE
L22           10 DUP REMOVE L21 (0 DUPLICATES REMOVED)
L23        21076 S L16 AND INTRACELLULAR
L24            0 S L23 AND CLYCOSPORIN A
L25           64 S L23 AND FK506
L26           32 DUP REMOVE L25 (32 DUPLICATES REMOVED)

=> s l23 and binding affinity
L27          272 L23 AND BINDING AFFINITY

=> s l27 and "10-4M"
L28            0 L27 AND "10-4M"

=> s l27 and high binding affinity
L29            6 L27 AND HIGH BINDING AFFINITY

=> dup remove l29
PROCESSING COMPLETED FOR L29
L30            3 DUP REMOVE L29 (3 DUPLICATES REMOVED)

=> d l30 1-3 cbib abs

L30  ANSWER 1 OF 3  EMBASE  COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
     on STN
2005056511  EMBASE  Magnetic resonance imaging visualization of targeted cells
     by the internalization of supramolecular adducts formed between avidin and
     biotinylated Gd(3+) chelates. Crich S.G.; Barge A.; Battistini E.; Cabella
     C.; Coluccia S.; Longo D.; Mainero V.; Tarone G.; Aime S.. S. Aime,
     Department of Chimica I.F.M., Universita di Torino, via P. Giuria 7,
     Turin, 10125, Italy. silvio.aime@unito.it. Journal of Biological Inorganic
     Chemistry  Vol. 10, No. 1, pp. 78-86  2005.
     Refs: 32.
     ISSN: 0949-8257. CODEN: JJBCFA
     Pub. Country: Germany. Language: English. Summary Language: English.
ED   Entered STN: 20050218
AB   The high binding affinity between avidin and
     biotin has been exploited to develop a procedure for magnetic resonance
     imaging (MRI) visualization of target cells.  SHIN3 and PANC1 tumor cell
     lines have been used as target cells because they possess on their
     membranes galactosyl receptors able to bind avidin molecules.  Avidin-Gd
     chelate adducts have been built by using two Gd complexes containing one
     (Gd-I) and two (Gd-II) biotin residues, respectively.  The relaxivities of
     such supramolecular adducts are significantly higher than those shown by
     free Gd-I and Gd-II.  There is evidence of the occurrence of multilayered
     adducts in which the bis-biotinylated Gd(3+) complex acts as a bridge
     between adjacent avidin molecules.  MRI differentiation of labeled versus
     unlabeled cells has been attained when approximately 6x10 (8) Gd units
     were internalized in each cell.  Furthermore, there is a marked decrease
     in the measured intracellular T(1) relaxivity as the number of
     internalized Gd complexes increases, probably owing to too short
     relaxation times of endosomic water protons with respect to their
     diffusion life-time. .COPYRGT. SBIC 2004.

L30  ANSWER 2 OF 3  CAPLUS  COPYRIGHT 2005 ACS on STN
2001:240695  Document No. 135:70605  Mechanism of specific nuclear transport
     of adriamycin: the mode of nuclear translocation of adriamycin-proteasome
     complex.  Kiyomiya, Ken-Ichi; Matsuo, Saburou; Kurebe, Masaru (Department
     of Toxicology, School of Veterinary Medicine, Osaka Prefecture University,
     Osaka, 599-8531, Japan).  Cancer Research, 61(6), 2467-2471 (English)
     2001.  CODEN: CNREA8.  ISSN: 0008-5472.  Publisher: American Association
     for Cancer Research.
```

AB Adriamycin (ADM), an anthracycline anticancer agent, is selectively stored in the nuclei of a variety of proliferating cells, but the precise mechanism of specific nuclear transport of ADM is not well known. Recently, the authors demonstrated that ADM shows **high binding affinity** to the cytoplasmic proteasomes of L1210 mouse leukemia cells and that taken up ADM by the cells selectively binds to proteasomes. Nuclear **targeting** of proteasome in proliferating cells may be mediated by the nuclear localization signals that are found in several of the α-type subunits of the 20S proteasome. To confirm nuclear transport of the ADM-proteasome complex, the authors synthesized a photoactive ADM analog, N-(p-azidobenzoyl)-ADM, and generated a photoaffinity-labeled proteasome complex. The 26S proteasome purified from the cytosol of L1210 cells had a high affinity to N-(p-azidobenzoyl)-ADM. SDS-PAGE anal. of the photoaffinity-labeled proteasome showed that low mol. weight bands (.apprx.21-31 kDa) of 20S proteasome had the highest photoaffinity. The photoaffinity-labeled proteasome was distributed in the cytoplasm and nuclei of digitonin-permeabilized L1210 and B-16 mouse melanoma cells in the presence of the cytosolic fraction and ATP. The rate of nuclear translocation of the proteasome was low in the absence of ATP. These results suggest that the proteasome is a specific translocator of ADM from the cytoplasm to the nucleus and that 20S proteasome components are the dominant ADM-binding sites. The nuclear transport of ADM-proteasome complex is regulated by an ATP-dependent nuclear pore-mediated mechanism.

L30 ANSWER 3 OF 3 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN DUPLICATE 1
2000374293 EMBASE Receptor-specific delivery of liposomes via folate-PEG-Chol. Guo W.; Lee T.; Sudimack J.; Lee R.J.. R.J. Lee, Div. Pharmaceut./Pharmaceut. Chem., College of Pharmacy, The Ohio State University, Columbus, OH 43210, United States. lee.1339@osu.edu. Journal of Liposome Research Vol. 10, No. 2-3, pp. 179-195 2000.
Refs: 29.
ISSN: 0898-2104. CODEN: JLREE7
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20001116
AB A novel lipophilic conjugate of folate, folate-PEG-Chol, was synthesized and evaluated for receptor-mediated **targeting** of liposomes to tumor cells. Liposomes composed of DSPC/Chol/PEG-DSPE/folate-PEG-Chol (60/34/5/1, m/m) were taken up by cultured folate receptor-bearing KB cells via a saturable mechanism. Cellular binding of these liposomes could be competitively inhibited by free folic acid with an IC50 of 0.39 mM, indicating an extraordinarily **high binding affinity.** Fluorescence micrographs of KB cells treated with targeted liposomes encapsulating calcein showed that they were distributed both on the cell surface and in **intracellular** vesicular compartments. Targeted liposomes carrying doxorubicin were shown to be 38 times more toxic to KB cells than non-targeted control liposomes. A biodistribution study in receptor-positive tumor-bearing C57BL/6 mice showed no significant differences between the tumor uptake of folate-PEG-liposomes and non-targeted control liposomes. This study has demonstrated that cholesterol could be used as an alternative to phospholipids as an effective anchor for incorporation of a **targeting** ligand into liposomes.

```
=> s l27 and low affinity
L31           6 L27 AND LOW AFFINITY

=> dup remove l31
PROCESSING COMPLETED FOR L31
L32           6 DUP REMOVE L31 (0 DUPLICATES REMOVED)

=> d l32 1-6 cbib abs
```

L32 ANSWER 1 OF 6 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2004352643 EMBASE Enhanced **intracellular** stability of sFv-F(c) fusion intrabodies. Strube R.W.; Chen S.-Y.. S.-Y. Chen, Dept. of Molec. and Human Genetics, Center for Cell and Gene Therapy, Baylor College of Medicine, 77030, Houston, TX, United States. sychen@bcm.tmc.edu. Methods Vol. 34, No. 2, pp. 179-183 2004.
Refs: 25.
ISSN: 1046-2023. CODEN: MTHDE
S 1046-2023(04)00072-6. Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20040902

AB The ability of **intracellular** antibodies (intrabodies) to block the function of a target protein can be dependent on the stability of the single-chain antibody (sFv) when expressed in the **intracellular** environment. **Low-affinity** sFvs capable of reaching high steady-state levels can be more effective modulators of target proteins than high-affinity, unstable sFvs. In an effort to enhance the **intracellular** stability of sFvs when expressed as intrabodies, we have generated novel sFv-F(c) fusion intrabodies. Fusion of the native sFv sequence with the entire heavy chain constant region fragment of IgG results in decreased turnover of the intrabody and enhanced steady-state accumulation of sFv-F(c) protein, while maintaining the ability to target intrabody expression to sub-cellular compartments. Here, we describe the rationale and design for this strategy using two anti-cyclin E sFvs constructed for use as intrabodies.

L32 ANSWER 2 OF 6 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2002313284 EMBASE Phosphatidylinositol 3-phosphate-interacting domains in PIKfyve. Binding specificity and role in PIKfyve endomembrane localization. Sbrissa D.; Ikonomov O.C.; Shisheva A.. A. Shisheva, Dept. of Physiology, Wayne State Univ. School of Medicine, 540 E. Canfield Ave., Detroit, MI 48201, United States. ashishev@med.wayne.edu. Journal of Biological Chemistry Vol. 277, No. 8, pp. 6073-6079 22 Feb 2002.
Refs: 37.
ISSN: 0021-9258. CODEN: JBCHA3
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20021003

AB PIKfyve is a phosphatidylinositol (PtdIns) 3-phosphate (P)-metabolizing enzyme, which, in addition to a C-terminally positioned catalytic domain, harbors several evolutionarily conserved domains, including a FYVE finger. The FYVE finger domains are thought to direct the protein localization to **intracellular** membrane PtdIns 3-P. Recent studies with several FYVE domain proteins challenge this general concept. Here we have examined the binding of PIKfyve's FYVE domain to PtdIns 3-P in vitro and in vivo and a plausible contribution of this binding mechanism for the **intracellular** localization of the full-length protein. We document now a specific and high affinity interaction of a recombinantly produced PIKfyve FYVE domain peptide fragment with PtdIns 3-P-containing liposomes that requires the presence of the conservative core of basic residues within the FYVE domain. PIKfyve localization to membranes of the late endocytic pathway was found to be absolutely dependent on the presence of an intact FYVE finger. Cell treatment with PI 3-kinase inhibitor wortmannin dissociated endosome-bound PIKfyve, indicating that the protein targeted the membrane PtdIns 3-P. An enzymatically inactive peptide fragment of the PIKfyve catalytic domain was found to also specifically bind to PtdIns 3-P-containing liposomes, with residue Lys-1999 being critical in the interaction. This binding, however, was of relatively **low affinity** and, in the cellular context, was found ineffective in directing the molecule to PtdIns 3-P-enriched endosomes. Collectively, these results demonstrate that interaction of the FYVE domain with PtdIns 3-P is absolutely necessary for PIKfyve **targeting** to the membranes of the late endocytic pathway and

determine PIKfyve as a downstream effector of PtdIns 3-P.

L32 ANSWER 3 OF 6 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2002349685 EMBASE Interferon-induced Mx proteins: Dynamin-like GTPases with antiviral activity. Haller O.; Kochs G.. O. Haller, Abteilung Virologie, Institut Med. Mikrobiologie/Hygiene, Universitat Freiburg, D-79008 Freiburg, Germany. Haller@ukl.uni-freiburg.de. Traffic Vol. 3, No. 10, pp. 710-717 2002.
Refs: 53.
ISSN: 1398-9219. CODEN: TRAFFA
Pub. Country: Denmark. Language: English. Summary Language: English.
ED Entered STN: 20021017
AB Mx proteins are interferon-induced GTPases that belong to the dynamin superfamily of large GTPases. Similarities include a high molecular weight, a propensity to self-assemble, a relatively **low affinity** for GTP, and a high intrinsic rate of GTP hydrolysis. A unique property of Mx GTPases is their antiviral activity against a wide range of RNA viruses, including bunya- and orthomyxoviruses. The human MxA GTPase accumulates in the cytoplasm of interferon-treated cells, partly associating with the endoplasmic reticulum. In the case of bunyaviruses, MxA interferes with transport of the viral nucleocapsid protein (N) to the Golgi compartment, the site of virus assembly. In the case of Thogoto virus (an orthomyxovirus), MxA prevents the incoming viral nucleocapsids from being transported into the nucleus, the site of viral transcription and replication. In both cases, the GTP-binding and carboxy-terminal effector functions of MxA are required for target recognition. In general, Mx GTPases appear to detect viral infection by sensing nucleocapsid-like structures. As a consequence, these viral components are trapped and sorted to locations where they become unavailable for the generation of new virus particles.

L32 ANSWER 4 OF 6 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
1999120850 EMBASE The PDE1-encoded **low-affinity** phosphodiesterase in the yeast Saccharomyces cerevisiae has a specific function in controlling agonist- induced cAMP signaling. Ma P.; Wera S.; Van Dijck P.; Thevelein J.M.. J.M. Thevelein, Lab. voor Moleculaire Celbiologie, Katholieke Universiteit Leuven, B-3001 Leuven-Heverlee, Flanders, Belgium. Molecular Biology of the Cell Vol. 10, No. 1, pp. 91-104 1999.
Refs: 44.
ISSN: 1059-1524. CODEN: MBCEEV
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 19990422
AB The yeast Saccharomyces cerevisiae contains two genes, PDE1 and PDE2, which respectively encode a **low-affinity** and a high-affinity cAMP phosphodiesterase: The physiological function of the **low-affinity** enzyme Pde1 is unclear. We show that deletion of PDE1, but not PDE2, results in a much higher cAMP accumulation upon addition of glucose or upon **intracellular** acidification. Overexpression of PDE1, but not PDE2, abolished the agonist- induced cAMP increases. These results indicate a specific role for Pde1 in controlling glucose and **intracellular** acidification-induced cAMP signaling. Elimination of a putative protein kinase A (PKA) phosphorylation site by mutagenesis of serine252 into alanine resulted in a Pde1(ala252) allele that apparently had reduced activity in vivo. Its presence in a wild-type strain partially enhanced the agonist-induced cAMP increases compared with pde1Δ. The difference between the Pde1(ala252) allele and wild-type Pde1 was strongly dependent on PKA activity. In a RAS2(val19) pde2Δ background, the Pde1(ala252) allele caused nearly the same hyperaccumulation of cAMP as pde1Δ, while its expression in a PKA-attenuated strain caused the same reduction in cAMP hyperaccumulation as wild-type Pde1. These results suggest that serine252 might be the first target site for feedback inhibition of cAMP accumulation by PKA. We

show that pde1 is rapidly phosphorylated in vivo upon addition of glucose to glycerol-grown cells, and this activation is absent in the Pde1(ala252) mutant. Pde1 belongs to a separate class of phosphodiesterases and is the first member shown to be phosphorylated. However, in vitro the Pde1(ala252) enzyme had the same catalytic activity as wild-type Pde1, both in crude extracts and after extensive purification. This indicates that the effects of the S252A mutation are not caused by simple inactivation of the enzyme. In vitro phosphorylation of Pde1 resulted in a modest and variable increase in activity, but only in crude extracts. This was absent in Pde1(ala252), and phosphate incorporation was strongly reduced. Apparently, phosphorylation of Pde1 does not change its intrinsic activity or affinity for cAMP but appears to be important in vivo for protein-protein interaction or for **targeting** Pde1 to a specific subcellular location. The PKA recognition site is conserved in the corresponding region of the Schizosaccharomyces pombe and Candida albicans Pde1 homologues, possibly indicating a similar control by phosphorylation.

L32 ANSWER 5 OF 6 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

1998272921 EMBASE The PH domain and the polybasic c domain of cytohesin-1 cooperate specifically in plasma membrane association and cellular function. Nagel W.; Schilcher P.; Zeitlmann L.; Kolanus W.. W. Kolanus, Laboratorium fur Molekulare Biologie, Genzentrum der Universitat Munchen, D-81377 Munchen, Germany. kolanus@lmb.uni-muenchen.de. Molecular Biology of the Cell Vol. 9, No. 8, pp. 1981-1994 1998.
Refs: 48.
ISSN: 1059-1524. CODEN: MBCEEV
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 19980924

AB Recruitment of **intracellular** proteins to the plasma membrane is a commonly found requirement for the initiation of signal transduction events. The recently discovered pleckstrin homology (PH) domain, a structurally conserved element found in .apprx.100 signaling proteins, has been implicated in this function, because some PH domains have been described to be involved in plasma membrane association. Furthermore, several PH domains bind to the phosphoinositides phosphatidylinositol-(4,5)-bisphosphate and phosphatidylinositol-(3,4,5)trisphosphate in vitro, however, mostly with **low affinity.** It is unclear how such weak interactions can be responsible for observed membrane binding in vivo as well as the resulting biological phenomena. Here, we investigate the structural and functional requirements for membrane association of cytohesin-1, a recently discovered regulatory protein of T cell adhesion. We demonstrate that both the PH domain and the adjacent carboxyl-terminal polybasic sequence of cytohesin-1 (c domain) are necessary for plasma membrane association and biological function, namely interference with Jurkat cell adhesion to intercellular adhesion molecule 1. Biosensor measurements revealed that phosphatidylinositol-(3,4,5)- trisphosphate binds to the PH domain and c domain together with high affinity (100 nM), whereas the isolated PH domain has a substantially lower affinity (2-3 μM). The cooperativity of both elements appears specific, because a chimeric protein, consisting of the c domain of cytohesin-1 and the PH domain of the β-adrenergic receptor kinase does not associate with membranes, nor does it inhibit adhesion. Moreover, replacement of the c domain of cytohesin- 1 with a palmitoylation-isoprenylation motif partially restored the biological function, but the specific **targeting** to the plasma membrane was not retained. Thus we conclude that two elements of cytohesin-1, the PH domain and the c domain, are required and sufficient for membrane association. This appears to be a common mechanism for plasma membrane **targeting** of PH domains, because we observed a similar functional cooperativity of the PH domain of Bruton's tyrosine kinase with the adjacent Bruton's tyrosine kinase motif, a novel zinc-containing fold.

L32 ANSWER 6 OF 6 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.

on STN

94046017 EMBASE Document No.: 1994046017. Receptor mediated endocytosis and **intracellular** fate of interleukin 1. Solari R.; Smithers N.; Kennard N.; Ray K.; Grenfell S.. Div. of Cellular and Molecular Sci., Glaxo Group Research,Greenford, Middlesex UB6 0HE, United Kingdom. Biochemical Pharmacology Vol. 47, No. 1, pp. 93-101 1994. ISSN: 0006-2952. CODEN: BCPCA6 Pub. Country: United Kingdom. Language: English. Summary Language: English.

ED Entered STN: 940306

AB We have studied the receptor mediated endocytosis of interleukin 1 (IL1) by the murine thymoma cell line EL4. These cells express the Type I IL1 receptor which binds its ligand with both high (K(d) = 65 pM) and **low affinity** (K(d) = 14.5 nM). We have shown that the two affinity states of the receptor have different rates of turnover both in the absence and presence of ligand. The biological responses of cells to IL1 stimulation are rapid and occur at low levels of receptor occupancy, whereas receptor mediated endocytosis of IL1 is relatively slow. Internalized IL1 appears to accumulate within cells in a non-degraded form and a proportion of this is associated with a detergent insoluble **intracellular** fraction, which may reflect transport to the nucleus. In this article, we review our previous findings and discuss the possible biological significance of IL1 internalization and nuclear **targeting.**

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	273.36	273.57

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-21.17	-21.17

STN INTERNATIONAL LOGOFF AT 12:19:14 ON 07 APR 2005

ANSWER 3 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
2000:894627 Document No. 134:142032 Cytotoxic derivatives of luteinizing hormone-releasing hormone (**LHRH**): Synthesis and evaluation. Rahimipour, Shai; Gescheidt, Georg; Mazur, Yehuda; Weiner, Lev; Koch, Yitzhak; Fridkin, Mati (Department of Organic Chemistry, Weizmann Institute of Science, Rehovot, 76100, Israel). Peptides for the New Millennium, Proceedings of the American Peptide Symposium, 16th, Minneapolis, MN, United States, June 26-July 1, 1999, Meeting Date 1999, 238-239. Editor(s): Fields, Gregg B.; Tam, James P.; Barany, George. Kluwer Academic Publishers: Dordrecht, Neth. (English) 2000. CODEN: 69ATHX.

AB The authors' briefly present findings from their study on cytotoxic derivs. of LH-releasing hormone (**LHRH**). The objectives of the study were focused mainly on (i) the design and synthesis of targeted chemotherapeutic compds. against cancer cells that overexpress **LHRH** receptors and (ii) to evaluate their physicochem. properties as well as their biol. activity. The studies confirmed that these compds. could be readily reduced to the corresponding semiquinones, which have a long lifetime. The **binding affinities** of different [D-Lys6]-**LHRH** cytotoxic derivs. to pituitary **LHRH** receptors were compared by competitive binding expts. All the conjugates demonstrated a rather high **binding affinity** to the **LHRH** receptors (2.8 nM-20 nM), although, somewhat reduced as compared to that of [D-Lys6]-**LHRH** (1.4 nM). The LH-releasing potencies of the [D-Lys6]-**LHRH** and its cytotoxic conjugates were evaluated in primary pituitary cell cultures. The expts. confirmed that all the analogs preserved their agonistic behavior similarly to their parent peptide-[D-Lys6]-**LHRH.** The cytotoxicity studies showed that the cytotoxic conjugates are more toxic (0.1 .mu.**M**) to the mouse pituitary cell line (αT3-1, possessing **LHRH** receptors), as compared to that of the cytotoxic moieties alone (1 μ **M**). Moreover, the cytotoxicity of the conjugated **LHRH** could be diminished by coadministration of [D-Lys6]-**LHRH**, which compete for **LHRH** binding sites. These results strongly suggest that the resulted toxicity is receptor mediated, and therefore these analogs can potentially be used for targeted chemotherapy.

L7 ANSWER 4 OF 37 EMBASE on STN
2000297257 EMBASE Role of protein kinase C in facilitation of luteinizing hormone (LH)-releasing hormone-induced LH surges by neuropeptide Y. Leupen S.M.; Levine J.E.. Dr. J.E. Levine, Hogan Hall 2-160, 2153 North Campus Drive, Evanston, IL 60208, United States. jlevine@nwu.edu. Endocrinology Vol. 140, No. 8, pp. 3682-3687 1999.
Refs: 43.
ISSN: 0013-7227. CODEN: ENDOAO
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20000914

AB In female rats, neuropeptide Y (NPY) facilitates **LHRH**-induced LH surges without affecting basal LH release. The signal transduction mechanisms mediating this facilitation are unknown. Here, the involvement of PKC in this process was investigated. Anterior pituitaries (APs) were removed from rats at 1400 h proestrus and perifused in vitro with M199 for 5 h. Affer an equilibration and baseline period, tissue received hourly 5-minute pulses of the PKC inhibitor GF109203X (GFX), 2.5 .mu.**M**, followed 15 min later by a 5-minute pulse of **LHRH** (10-8 **M**), NPY (10-6 **M**), or phorbol 12-myristate 13-acetate (PMA, 50 nM), or some combination. This regimen was repeated hourly for 3 h. As shown previously, NPY had no effect on basal LH release but greatly facilitated **LHRH**-induced LH release. Treatment with PMA also facilitated **LHRH**-induced LH release, to approximately the same degree as NPY. Inhibition of PKC activity with GFX completely prevented NPY's and PMA's facilitation of LH release but did not inhibit LH release stimulated by **LHRH** alone. Because previous work suggested

involvement of both NPY and PKC in alterations of **LHRH** receptor affinity or number, the in vivo effects of NPY on **LHRH** binding characteristics were also investigated. Although NPY treatment reliably enhanced **LHRH**-induced LH and FSH surges in proestrous rats, this action was not accompanied by any detectable change in the affinity or concentration of **LHRH** receptors in anterior pituitary cell membranes. In summary, we have found that NPY's actions are blocked by PKC inhibition, mimicked by PKC stimulation, and not associated with any overt alterations in **LHRH** receptor affinity or number. We conclude that PKC activation is required for NPY's facilitation of **LHRH**-induced LH surges, and that this mechanism likely involves PKC targets other than those which may alter **LHRH** receptor number or affinity.

L7 ANSWER 5 OF 37 MEDLINE on STN DUPLICATE 3
1998141763. PubMed ID: 9482659. Addition of catfish gonadotropin-releasing hormone (GnRH) receptor intracellular carboxyl-terminal tail to rat GnRH receptor alters receptor expression and regulation. Lin X; Janovick J A; Brothers S; Blomenrohr M; Bogerd J; Conn P M. (Oregon Regional Primate Research Center, Beaverton 97006, USA.) Molecular endocrinology (Baltimore, Md.), (1998 Feb) 12 (2) 161-71. Journal code: 8801431. ISSN: 0888-8809. Pub. country: United States. Language: English.

AB Mammalian GnRH receptor (GnRHR) is unique among G protein-coupled seven-transmembrane segment receptors due to the absence of an intracellular C-terminal tail frequently important for internalization and/or desensitization of other G protein-coupled receptors. The recent cloning of nonmammalian (i.e. catfish, goldfish, frog, and chicken) GnRHRs shows that these contain an intracellular C terminus. Addition of the 51-amino acid intracellular C terminus from catfish GnRHR (cfGnRHR) to rat GnRHR (rGnRHR) did not affect rGnRHR **binding affinity** but elevated receptor expression by about 5-fold. Truncation of the added C terminus impaired the elevated receptor-binding sites by 3- to 8-fold, depending on the truncation site. In addition, introducing the C terminus to rGnRHR altered the pattern of receptor regulation from biphasic down-regulation and recovery to monophasic down-regulation. The extent of down-regulation was also enhanced. The alteration in receptor regulation due to the addition of a C terminus was reversed by truncation of the added C terminus. Furthermore, addition of the cfGnRHR C terminus to rGnRHR significantly augmented the inositol phospholipid (IP) response of transfected cells to Buserelin, but this did not result from the elevation of receptor-binding sites. Addition of the C terminus did not affect Buserelin-stimulated cAMP and PRL release. GH3 cells transfected with wild-type cfGnRHR did not show measurable Buserelin binding or significant stimulation of IP, cAMP, or PRL in response to Buserelin (10[-13]-10[-9] **M**). GH3 cells transfected with C terminus-truncated cfGnRHR showed no IP response to Buserelin (10[-13]-10[-7] **M**). These results suggest that addition of the cfGnRHR intracellular C terminus to rGnRHR has a significant impact on rGnRHR expression and regulation and efficiency of differential receptor coupling to G proteins.

L7 ANSWER 6 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
1998:90206 Document No. 128:226368 Structure elucidation and conformational analysis of gonadotropin releasing hormone and its novel synthetic analog [Tyr(OMe)5,D-Lys6,Aze9NHEt]GnRH: the importance of aromatic clustering in the receptor binding activity. Matsoukas, J. M.; Keramida, M.; Panagiotopoulos, D.; Mavromoustakos, T.; Maia, H. L. S.; Bigam, G.; Pati, D.; Habibi, H. R.; Moore, G. J. (Department of Chemistry, University of Patras, Patras, Greece). European Journal of Medicinal Chemistry, 32(12), 927-940 (English) 1997. CODEN: EJMCA5. ISSN: 0223-5234. Publisher: Editions Scientifiques et Medicales Elsevier.

AB The conformational properties of gonadotropin releasing hormone (GnRH) in dimethylsulfoxide -d6 were investigated by nuclear Overhauser effect (nOe) enhancements studies and were compared with the conformational properties of its analog [Tyr(OMe)5]GnRH resulting after methylation of the tyrosine

hydroxyl. Assignment of all backbone and side-chain protons was possible by combining information from intraresidue nOe studies with two-dimensional correlated spectroscopy (COSY/TOCSY) studies. Saturation of distinct proton resonances of the three aromatic residues Tyr, His, Trp, in clear areas of the NMR spectrum of GnRH resulted in inter-residue enhancements of aromatic resonances indicating the proximity of the three aromatic rings. This spatial proximity is not observed in [Tyr(OMe)5]GnRH and is correlated with a lower receptor **binding affinity** in the rat pituitary (Kd = 1.53 X 10-6 **M**) compared with that exerted by GnRH (Kd = 3.69 X 10-9 **M**). However, substitution of Gly at position 6 of [Tyr(OMe)5]GnRH with D-Lys6 and further replacement of Pro at position 9 with the more rigid Aze residue [Tyr(OMe)5, D-Lys6, Aze9NHEt]GnRH significantly improved the **binding affinity** (Kd = 0.689 X 10-9) and this may be due to the restoration of the ring cluster. Overall, the clustering of the aromatic rings observed in GnRH was not seen in [Tyr(OMe)5]GnRH and this conformational difference may be responsible for receptor recognition and higher binding of the parent peptide.

L7 ANSWER 7 OF 37 EMBASE
on STN

95158867 EMBASE Document No.: 1995158867. New pseudononapeptide bombesin antagonists with C-terminal LeuΨ(CH2N)Tac-NH2 show high **binding affinity** to bombesin/GRP receptors on CFPAC-1 human pancreatic cancer cells. Cai R.-Z.; Qin Y.; Ertl T.; Schally A.V.. Veterans Affairs Medical Ctr. (151), 1601 Perdido Street,New Orleans, LA 70146, United States. International Journal of Oncology Vol. 6, No. 6, pp. 1165-1172 1995.
ISSN: 1019-6439. CODEN: IJONES
Pub. Country: Greece. Language: English. Summary Language: English.

ED Entered STN: 950619

AB It has been demonstrated that bombesin/GRP antagonist D-Tpi6,Leu13Ψ(CH2NH) Leu14-BN(6-14) (RC-3095) inhibits effectively the growth of pancreatic cancer and other tumors in experimental animals and in cell cultures. In an attempt to develop antagonists with still greater antitumor activity, several new pseudononapeptide bombesin/GRP antagonists containing C-terminal LeuΨ(CH2N)Tac-NH2 have been synthesized in our laboratory. In this study, we investigated the ability of four Leu13Ψ(CH2N)Tac14-BN(6-14) antagonists to inhibit the binding of bombesin to specific receptors for bombesin/GRP on CFPAC-1 human pancreatic cancer cells. Receptor binding assays were performed by incubating CFPAC-1 cells (5 x 104 cells/well) with 0.5 nM [125I]-Tyr4-bombesin in the absence or presence of (1 pM to 10 μ **M**) unlabeled bombesin, GRP(14-27) and various antagonists for 2 h at 22°C. Displacement assays showed that antagonist D-Tpi6,Leu13Ψ(CH2N)Tac14-BN(6-14) (RC-3910-II) with a similar structure to RC-3095, but a different C-terminal, had a **binding affinity** to CFPAC-1 cells 15 times higher than RC-3095. Three other antagonists, RC-3925-II, RC-3940-II and RC-3950-II contained the same C-terminal LeuΨ(CH2N)Tac-NH2 as RC-3910-II, but had different N-terminal residues: D-Cpa, Hca and D-Phe, respectively. Among them, Hca6,Leu13Ψ(CH2N)Tac14-BN(6-14) (RC-3940-II) showed the highest **binding affinity** to the receptors on CFPAC-1 cells, which was 50 times higher than that of RC-3095 or 3 times greater than RC-3910-II. Our findings suggest the merit of further investigation of pseudononapeptide bombesin/GRP antagonist RC-3940-II and related analogs for a possible development of a new hormonal therapy for pancreatic cancer.

L7 ANSWER 8 OF 37 MEDLINE on STN DUPLICATE 4

94283274. PubMed ID: 8013367. Stable transfection of GH3 cells with rat gonadotropin-releasing hormone receptor complementary deoxyribonucleic acid results in expression of a receptor coupled to cyclic adenosine 3',5'-monophosphate-dependent prolactin release via a G-protein. Kuphal D;

Janovick J A; Kaiser U B; Chin W W; Conn P M. (Department of Pharmacology, University of Iowa College of Medicine, Iowa City 52242-1109.) Endocrinology, (1994 Jul) 135 (1) 315-20. Journal code: 0375040. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB GH3 cells, which normally release PRL in response to stimulation by TRH, have been stably transfected with rat GnRH receptor complementary DNA (GGH3-1' cells). Unlike the parent line, GGH3-1' cells express GnRH receptor, which can be measured in a radioligand assay using a metabolically stable GnRH analog. The number of receptors (11,000 +/- 2,800 receptors/cell; n = 3) and Kd (4.1 +/- 1.0 x 10(-8) **M**; n = 3), determined using a radioiodinated GnRH agonist, as well as binding inhibition values for GnRH agonists and antagonists and for unrelated substances suggest that this receptor is similar to those expressed in cell cultures derived from rat pituitaries, although the **binding affinity** is about 1 log lower in the former. Unlike GnRH-stimulated release of gonadotropins from primary pituitary cultures, which does not require protein synthesis and is not coupled to cAMP production, GnRH-stimulated PRL release from the transfected cell line is absolutely dependent on protein synthesis, and cAMP fulfills the requirements of a second messenger. The receptor appears to be coupled to adenylate cyclase-mediated PRL release through a cholera toxin-sensitive G-protein. These studies provide functional evidence to support the view that the cloned receptor is the physiological receptor for the releasing hormone, and that this receptor can differentially couple to G-proteins depending on their availability and accessibility in the target cell.

L7 ANSWER 9 OF 37 EMBASE on STN

94207500 EMBASE Document No.: 1994207500. Acute increase in responsiveness of luteinizing hormone (LH)-releasing hormone nerve terminals to neuropeptide-Y stimulation before the preovulatory LH surge. Besecke L.M.; Levine J.E.. Neurobiology/Physiology Department, 2153 Sheridan Road,Evanston, IL 60208, United States. Endocrinology Vol. 135, No. 1, pp. 63-66 1994.
ISSN: 0013-7227. CODEN: ENDOAO
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 940727

AB Neuropeptide-Y (NPY) neurons regulate LH secretion in part through facilitation of **LHRH** release. We tested the hypothesis that responsiveness of **LHRH** neurons to NPY's facilitatory actions is physiologically regulated during the estrous cycle, and specifically, that it may be increased as a component of the gonadotropin surge-generating process. A dynamic superfusion paradigm was used to examine the role of cycle stage and time of day on **LHRH** responsiveness to NPY stimulation, using median eminence tissue from animals killed at 0900, 1400, and 1800 h on metestrus and proestrus. Tissue obtained at 0900 and 1800 h on metestrus did not exhibit significant **LHRH** responses to 10-7 **M** NPY, and only moderate responses were seen at 1400 h on metestrus and 0900 h on proestrus. At 1400 h on proestrus, however, median eminence responsiveness to the same concentration of NPY was significantly increased, with **LHRH** responses to NPY being 2- to 5-fold greater than those at 0900 ($P < 0.01$), 1400 ($P < 0.05$), and 1800 h on metestrus ($P < 0.01$) and at 0900 h on proestrus ($P < 0.05$). Neither cycle-related changes in basal **LHRH** release nor changes in the releasability of **LHRH** in response to depolarization could account for the accentuated responses in the 1400 h proestrus group. These data clearly demonstrate that the responsiveness of **LHRH** terminals and/or their afferents to the actions of NPY is acutely enhanced during a brief window of time on proestrus, viz. immediately before generation of gonadotropin surges. Our findings are consistent with the hypothesis that the preovulatory endocrine milieu permits an acute increase in the responsiveness of **LHRH** nerve terminals to the actions of NPY, perhaps by prompting increases in the number and/or affinity of NPY receptors.

L7 ANSWER 10 OF 37 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

94360490 EMBASE Document No.: 1994360490. Dynorphin binds to neuropeptide Y and peptide YY receptors in human neuroblastoma cell lines. Miura M.; Inui A.; Sano K.; Ueno N.; Teranishi A.; Hirosue Y.; Nakajima M.; Okita M.; Togami J.; Koshiya K.; Baba S.; Kasuga M.. Second Dept. of Internal Medicine, Kobe University School of Medicine, Kusunoki-cho,Chuo-ku, Kobe 650, Japan. American Journal of Physiology - Endocrinology and Metabolism Vol. 267, No. 5 30-5, pp. E702-E709 1994.
ISSN: 0193-1849. CODEN: AJPMD
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 941229

AB The modulation of neuropeptide Y (NPY) and peptide Y (PYY) receptors by dynorphin, luteinizing hormone-releasing hormone (**LHRH**), corticotropin- releasing factor (CRF), and cholecystokinin octapeptide has been studied in human neuroblastoma cell lines SK-N-MC and SMS-MSN, which express Y1 and Y2 receptors for NPY/PYY. Dynorphin A and **LHRH** inhibited the binding of NPY/PYY to SK-N-MC cell membranes at concentrations ranging from 10-7 to 10-5 **M**, whereas dynorphin A and CRF were effective in SMS-MSN cells. The inhibitory effect of dynorphin A on NPY/PYY binding was observed in the presence of guanosine 5'-O-(3-thiotriphosphate), a nonhydrolyzable GTP analogue, as well as H-7 and H-8, novel inhibitors of protein kinases C and A. However, U- 50488, the most potent κ-selective compound did not mimic the dynorphin action. Dynorphin A showed neither effect on the dissociation of NPY/PYY from their receptors nor inhibition on the basal as well as forskolin-stimulated adenosine 3',5'-cyclic monophosphate response. These results indicate that the interaction of dynorphin A with Y1 and Y2 receptors is not mediated by changes in receptor-G protein interaction, receptor phosphorylation, and allosteric binding to NPY/PYY receptors but that dynorphin A binds to NPY/PYY receptors at high concentrations, probably in an antagonistic manner.

L7 ANSWER 11 OF 37 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

93342179 EMBASE Document No.: 1993342179. High affinity binding and direct antiproliferative effects of **LHRH** analogues in human ovarian cancer cell lines. Emons G.; Ortmann O.; Becker M.; Irmer G.; Springer B.; Laun R.; Holzel F.; Schulz K.-D.; Schally A.V.. Department of Obstetrics/Gynecology, Philipps-University, Pilgrimstein 3,D-35037 Marburg, Germany. Cancer Research Vol. 53, No. 22, pp. 5439-5446 1993.
ISSN: 0008-5472. CODEN: CNREA8
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 931226

AB Recently, specific binding sites for luteinizing hormone releasing hormone (**LHRH**) and its analogues have been demonstrated in biopsy samples of human epithelial ovarian cancer. Their biological significance remained obscure. In this study we ascertained whether such **LHRH** -binding sites are also present in the human epithelial ovarian cancer cell lines EFO-21 and EFO-27 and if they could mediate antiproliferative effects of **LHRH** analogues. Using [125I, D- Trp6]**LHRH**, a high affinity/low capacity binding site was detected in both lines: EFO-21 (Kd1 = 1.5 x 10-9 **M**; binding capacity (Bmax1) = 4.9 fmol/106 cells) and EFO-27 (Kd1 = 1.7 x 10-9 **M**; Bmax1 = 3 fmol/106 cells). In addition, a second class of low affinity/high capacity binding sites (EFO-21: Kd2 = 7.5 x 10-6 **M**; Bmax2 = 24 pmol/106 cells; EFO-27: Kd2 = 4.3 x 10-6 **M**; Bmax2 = 14.5 pmol/106 cells) was demonstrated. Specific binding of [125I, D-Trp6] **LHRH** was displaced with nearly equal efficiency by unlabeled [D-Trp6]**LHRH**, the **LHRH**-antagonists SB-75 and Hoe- 013, and by native **LHRH** but not by unrelated peptides such as oxytocin and somatostatin. In the presence of 10-5 **M** agonist [D-Trp6]**LHRH**, the proliferation of both cell lines was

significantly reduced to 77% of controls after 24 h and to approx. 60% after 6 days. Lower concentrations (10-9 **M**) of the agonist, significantly decreased the proliferation to 87.5% for EFO- 21 and 86% for EFO-27 after 6 days. These antiproliferative effects were enhanced by increasing doses of [D-Trp6]**LHRH** and were maximal at 10-5 **M** (EFO-21: 65.5% of control, EFO-27: 68% of control). Similar dose-dependent antiproliferative effects were obtained in EFO-21 line with the **LHRH**- antagonists SB-75 and Hoe-013, while these analogues had no effects on the proliferation of EFO-27 cells. SB-75 partly antagonized the antiproliferative effect of [D-Trp6]**LHRH** in a dose dependent way in the EFO-27 line. These data suggest that **LHRH** analogues can directly inhibit the in vitro proliferation of human ovarian cancer cells. This effect might be mediated through the high affinity **LHRH** binding sites.

L7 ANSWER 12 OF 37 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

92355116 EMBASE Document No.: 1992355116. Characterization of gonadotropin-releasing hormone binding to pituitary receptors in the gilthead seabream (Sparus aurata). Pagelson G.; Zohar Y.. Center of Marine Biotechnology, University of Maryland, 600 E. Lombard Street,Baltimore, MD 21202, United States. Biology of Reproduction Vol. 47, No. 6, pp. 1004-1008 1992.
ISSN: 0006-3363. CODEN: BIREBV
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 921227

AB Receptors for GnRH in the pituitary of Sparus aurata were characterized using iodinated [D-Ala6-Pro9-NEt]-**LHRH** (GnRHa). Equilibrium binding of the ligand to the receptor was achieved after 1 h at 4°C. Binding of the radioligand was a function of tissue concentration, with a linear correlation over the range of one-sixteenth to three-fourths pituitary per tube. Displacement experiments with salmon GnRH (sGnRH), GnRHa, as well as unrelated peptides demonstrated the specificity of the receptors. Binding was found to be saturable at ligand concentrations of 4 x 10-9 **M**. Scatchard analysis of the saturation data suggested the presence of a single class of high-affinity sites (K(a) = 0.567 ± 0.136 x 109 **M**-1, B(max) = 1091 ± 207 fmol/mg protein).

L7 ANSWER 13 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN

1993:20006 Document No. 118:20006 Forskolin but not cholera toxin bypasses flufenamate-induced inhibition of cAMP production in anterior pituitaries. Bourne, Gregory A. (Dep. Physiol., Univ. Saskatchewan, Saskatoon, SK, S7N 0W0, Can.). Pharmacology & Toxicology (Oxford, United Kingdom), 71(5), 391-4 (English) 1992. CODEN: PHTOEH. ISSN: 0901-9928.

AB The pharmacol. activators of adenylyl cyclase (cholera toxin and forskolin) were utilized in the present study to determine whether they could bypass the inhibitory effects of flufenamate on cAMP production in rat hemipituitaries. During 2-h incubations, 10 .mu.**M** flufenamate inhibited gonadotropin-releasing hormone (GnRH)-stimulated (25 nM) cAMP production Flufenamate did not affect GnRH-receptor interactions as evidenced by its inability to significantly affect either the **binding affinity** or the binding capacity for GnRH. Addnl., flufenamate inhibited the cholera toxin-stimulated cAMP production, but was ineffective against forskolin-induced activation of adenylyl cyclase. Apparently, forskolin can be used to restore cAMP production in the presence of flufenamate. Since GnRH and cholera toxin stimulate cAMP production via the GnRH receptor and the Gs protein, resp., and forskolin exerts its stimulatory effects via the catalytic component, the data are consistent with the possibility that flufenamate exerts its inhibitory effect at the level of the Gs protein.

L7 ANSWER 14 OF 37 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 5

92:116226 The Genuine Article (R) Number: HE326. ACTIVITY OF VERTEBRATE GONADOTROPIN-RELEASING HORMONES AND ANALOGS WITH VARIANT AMINO-ACID-RESIDUES IN POSITION-5, POSITION-7 AND POSITION-8 IN THE GOLDFISH PITUITARY. HABIBI H R (Reprint); PETER R E; NAHORNIAK C S; MILTON R C D; MILLAR R P. UNIV CALGARY, DEPT BIOL SCI, CALGARY T2N 1N4, ALBERTA, CANADA (Reprint); UNIV ALBERTA, DEPT ZOOL, EDMONTON T6G 2E1, ALBERTA, CANADA; UNIV CAPE TOWN, SCH MED, DEPT CHEM PATHOL, REGULATORY PEPTIDES RES UNIT, CAPE TOWN, SOUTH AFRICA. REGULATORY PEPTIDES (18 FEB 1992) Vol. 37, No. 3, pp. 271-284. ISSN: 0167-0115. Pub. country: CANADA; SOUTH AFRICA. Language: ENGLISH.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

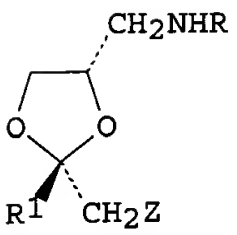
AB All non-mammalian vertebrates as well as marsupial mammals have two or more forms of gonadotropin-releasing hormone (GnRH) in the brain. Goldfish brain and pituitary contains two molecular forms of GnRH, salmon GnRH ([Trp7, Leu8]**m**-GnRH; s-GnRH) and chicken GnRH-II ([His5, Trp7, Tyr8]**m**-GnRH; cII-GnRH). Both sGnRH and cII-GnRH stimulate gonadotropin (GtH) as well as growth hormone (GH) release from the goldfish pituitary. The purpose of the present study was to study the activity of the five known forms of GnRHs as well as analogs of mammalian GnRH (**m**-GnRH) with variant amino acid residues in positions 5, 7 and 8 in terms of binding to GnRH receptors, and release of GTH and GH from the perifused fragments of goldfish pituitary in vitro. All five vertebrate GnRH peptides stimulated both GtH and GH release in a dose-dependent manner, although their potencies were very different. cII-GnRH was somewhat more active than s-GnRH in releasing GtH, whereas s-GnRH tended to have a greater potency than cII-GnRH in terms of GH release. Both chicken GnRH-I (cI-GnRH) and lamprey GnRH (l-GnRH) were significantly less potent than mGnRH, s-GnRH and cII-GnRH in releasing GtH and GH. cII-GnRH binds with higher affinity for the high affinity binding sites compared to all other native peptides. The activity of [Trp7]-GnRH was similar to both s-GnRH and cII-GnRH in releasing GtH and GH. Substitution of His5 resulted in a significant decrease in GtH releasing potencies compared to mGnRH, sGnRH and cII-GnRH. [His5]-GnRH also had lower GH releasing potency than mGnRH and sGnRH. Tyr8, His8 and Leu8 substitutions caused significant decreases in GtH releasing potencies compared to mGnRH, s-GnRH and cII-GnRH, but did not cause a significant change in GH releasing potency. The combination of [His5, Trp7]-GnRH had GtH and GH releasing activities similar to **m**-GnRH, s-GnRH and cII-GnRH. However, [His5, Tyr8]-GnRH had significantly lower GtH but not GH releasing potency compared to **m**-GnRH, s-GnRH and cII-GnRH. In terms of **binding affinity**, there were no correlations between **binding affinities** of the high or low affinity binding sites and GH or GtH releasing activities of the peptides used in the present study, suggesting that full molecular structure of native peptides are required for receptor recognition; in this context, previous studies demonstrated a good correlation between high affinity GnRH receptor binding and GTH release activity for GnRH analogs containing Trp7, Leu8 residues and various D-amino acids at position 6. Viewing all of the data together it is apparent that the presence of tryptophan in position 7 of the native GnRH peptides in goldfish, s-GnRH and cII-GnRH, is essential for the high potency of these peptides in releasing GtH from the goldfish pituitary. It is further evident that in goldfish the GH releasing activities of GnRH peptides are much less affected by position 8 substitutions than are the GtH releasing activities; position 8 substitutions in the **m**-GnRH molecule with histidine, leucine, methionine or tyrosine produces analogs that are significantly less potent in terms of GtH and are in this way more selective for the release of GH.

These findings support the hypothesis that the GnRH receptors on somatotrophs and gonadotrophs in goldfish are different and have different requirements for optimal ligand binding and activity.

L7 ANSWER 15 OF 37 EMBASE on STN

92235461 EMBASE Document No.: 1992235461. Antiproliferative effects of luteinizing hormone-releasing hormone agonists on the human prostatic cancer cell line LNCaP. Limonta P.; Dondi D.; Moretti R.M.; Maggi R.; Motta M.. Department of Endocrinology, University of Milan, Via Balzaretti 9,20133 Milan, Italy. Journal of Clinical Endocrinology and Metabolism Vol. 75, No. 1, pp. 207-212 1992.
ISSN: 0021-972X. CODEN: JCEMAZ
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 920830
AB Highly potent LH-releasing hormone (**LHRH**) agonists have been recently introduced in therapy for the treatment of the carcinoma of the prostate, an androgen-dependent pathology. These peptides are believed to act mainly by inhibiting the pituitary-testicular axis and, consequently, by reducing testosterone levels. The recent observation that binding sites for **LHRH** analogs are present on prostatic tumor tissue suggests that these drugs could also act directly on the tumor. To verify this hypothesis, the effects of two potent **LHRH** agonists [Zoladex (Z) and Buserelin (B)] have been studied on the proliferation of the human prostatic cancer cell line LNCaP (lymph node carcinoma of the prostate). LNCaP cells were treated for 9 days with different doses of either Z or B (concentrations from 10-12-10-6 **M**). Both analogs significantly inhibited cell proliferation at doses between 10- 9-10-6 **M**. The antiproliferative action of the two **LHRH** agonists was shown to be dose dependent, with IC50 values of 0.82 and 1.79 nM for Z and B, respectively. A similar treatment with B was without any significant effect on the proliferation of a mouse embryo fibroblast cell line (Swiss 3T3), which was used as a nontumoral control. The inhibitory action of both **LHRH** agonists (10-8 **M**) on LNCaP cell proliferation was completely antagonized by the simultaneous treatment of the cells with a potent **LHRH** antagonist (Nal- Arg-**LHRH** ; 10-8 **M**); when given alone at the dose selected, the antagonist did not affect cell growth. These results clearly suggest that the antiproliferative effect of **LHRH** agonists on LNCaP cells may be mediated by specific receptors. The presence of binding sites for [125I]B was consequently demonstrated on the membranes of LNCaP cells cultured in a medium containing charcoal-stripped fetal calf serum, i.e. in the absence of steroids. The affinity of these binding sites for the ligand was lower than that observed for the same receptors on rat pituitary membranes. To clarify the mechanism of the antiproliferative action of the **LHRH** agonists, the effects of both Z and B on the incorporation of [3H]thymidine and [14C]methionine into LNCaP cells were investigated. During a short incubation period (3 h), the two **LHRH** agonists rapidly inhibited [3H]thymidine incorporation into the cells. The same treatment did not affect the incorporation of [14C]methionine into proteins. It is concluded that 1) **LHRH** agonists specifically inhibit LNCaP cell proliferation in a dose-dependent manner; 2) the antiproliferative action of **LHRH** agonists is completely antagonized by simultaneous treatment of the cells with a potent **LHRH** antagonist; 3) LNCaP cells possess **LHRH** receptors; 4) **LHRH** agonists seem to interfere with thymidine (and possibly DNA) metabolism without affecting protein synthesis; and 5) it may be speculated that **LHRH** agonists, when used for the treatment of prostatic carcinoma, might inhibit tumor growth not only by reducing testosterone secretion (through suppression of the activity of the pituitary-testicular axis), but also by exerting a direct antiproliferative action at the level of the tumor.

L7 ANSWER 16 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
1991:492957 Document No. 115:92957 Preparation of N-[2-(imidazolomethyl)-4-dioxolanylmethyl]prolylhistidineamides and analogs as sex hormone suppressants. De, Biswanath; Jae, Hwan Soo; Plattner, Jacob J. (Abbott Laboratories, USA). U.S. US 4992421 A 19910212, 10 pp. (English).
CODEN: USXXAM. APPLICATION: US 1988-183201 19880419.
GI



I

AB The title compds. [I; R = COB; B = CH(NHR3)CH2R2, 2-phenylacetyl-3-oxocyclopentyl; R1 = (un)substituted Ph; R2 = heterocyclyl; R3 = H, CO2CMe3, CO2CH2Ph, COCH(NHR5)CH2R4, etc.; R4 = OH, heterocyclyl; R5 = H, CO2CMe3, CO2CH2Ph, etc.; Z = triazolo, imidazolo] were prepared as LH releasing hormone (**LHRH**) antagonists. Thus, I (R1 = 2,4-Cl2C6H3, Z = imidazolo) (II; R = H) (preparation given) was condensed with QOH [Q = N-[N-(2,2-diphenylethoxycarbonyl)prolyl]histidyl] (preparation described) to give II (R = Q) which had KI of 0.48 .mu.**M** for **LHRH** receptor **binding affinity.**

L7 ANSWER 17 OF 37 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 6

1991:229035 Document No.: PREV199191120495; BA91:120495. REGULATION OF **LHRH** RECEPTOR BINDING BY HETEROLOGOUS AND AUTOLOGOUS RECEPTOR-STIMULATED TYROSINE PHOSPHORYLATION. LIEBOW C [Reprint author]; LEE M T; KAMER A R; SCHALLY A V. DEP ORAL SURGERY, STATE UNIV NEW YORK AT BUFFALO SCH DENTAL MED, BUFFALO, NY 14214, USA. Proceedings of the National Academy of Sciences of the United States of America, (1991) Vol. 88, No. 6, pp. 2244-2248.
CODEN: PNASA6. ISSN: 0027-8424. Language: ENGLISH.

AB Pancreatic cancers overexpress tyrosine kinase and luteinizing hormone-release hormone (LH-RH) receptor (LH-RHR)-mediated tyrosine phosphatase. LH-RHR is a 60-kDa protein. One of the substrates of epidermal growth factor (EGF)-stimulted tyrosine kinase activity and LH-RH- and somatostatin-stimulated tyrosine phosphatase activity is also a 60-kDa protein. This suggests the possibility that LH-RHR regulation by tyrosine phosphatase and tyrosine kinase is mediated by (de)phosphorylation of existing LH-RHR. To test this hypothesis, membranes of MIA PaCa-2 cells, a human dedifferentiated pancreatic cancer cell line, were incubated without hormone (control) or with 0.1 μ **M** EGF or somatostatin analogue RC-160 for 1 hr at 4° C to phosphorylate the 60-kDa protein. Competition binding experiments with I125-labeled [D-Trp6]LH-RH by displacement with a nonradioactive ligand showed that the LH-RH binding in 69% of the points was increased by EGF and 85% was decreased by RC-160 compared with controls (n = 61; both significant, P < 0.001). The specific binding was altered, increasing 50-150% after preincubation with EGF and decreasing 60-70% after RC-160. No change was seen in the **binding affinity** constant after pretreatment with EGF or RC-160. This shows that phosphorylation regulates binding of LH-RH and may explain the up-regulation by EGF and down-regulation by RC-160 and by LH-RH of the LH-RH response.

L7 ANSWER 18 OF 37 MEDLINE on STN DUPLICATE 7

91183086. PubMed ID: 1849024. Homologous desensitization of gonadotropin-releasing hormone (GnRH) receptors in the goldfish pituitary: effects of native GnRH peptides and a synthetic GnRH antagonist. Habibi H R. (Department of Biological Sciences, University of Calgary, Alberta, Canada.) Biology of reproduction, (1991 Feb) 44 (2) 275-83. Journal code: 0207224. ISSN: 0006-3363. Pub. country: United States. Language: English.

AB Gonadotropin-releasing hormone (GnRH) stimulates release of gonadotropin hormone (GTH) through interaction with high affinity receptors in the

goldfish pituitary. In the present study, we investigated desensitization of two native GnRH peptides, [Trp7, Leu8]-GnRH (sGnRH) and [His5, Trp7, Tyr8]-GnRH (cGnRH-II), using superfused fragments of goldfish pituitary in vitro. Pulsatile treatment with either sGnRH or cGnRH-II (2-min pulses given every 60 min) resulted in dose-dependent secretion of GTH from the goldfish pituitary; cGnRH-II had a greater GTH release potency and displayed a greater receptor **binding affinity** than sGnRH. Both sGnRH and cGnRH-II-induced GTH release were partially inhibited by concomitant treatment with either [D-Phe2, Pro3, D-Phe6]-GnRH or [D-pGlu1, D-Phe2, D-Trp3.6]-GnRH. These antagonists had greater receptor **binding affinities** than the native peptides, with no stimulatory action on GTH release in the absence of the GnRH agonists. Continuous treatment with either sGnRH or cGnRH-II (10(-7) **M**), rapidly desensitized pituitary GTH release in a biphasic fashion; initially there was a rapid increase in GTH release of approximately 10-20-fold (phase 1), followed by a sharp decline in GTH release, reaching a stable concentration 2-3-fold above the basal level (phase 2). Further stimulation of the pituitaries with sGnRH or cGnRH-II (10(-7) **M**) (second treatment) after 60 min recovery resulted in a significantly lower sGnRH or cGnRH-II-induced GTH release compared to that observed during the initial treatment period. (ABSTRACT TRUNCATED AT 250 WORDS)

L7 ANSWER 19 OF 37 MEDLINE on STN

91152177. PubMed ID: 1963320. Photoaffinity labeling of pituitary gonadotropin-releasing hormone receptors in goldfish (Carassius auratus). Habibi H R; Peter R E; Hazum E. (Department of Biological Sciences, University of Calgary, Alberta, Canada.) Biology of reproduction, (1990 Dec) 43 (6) 1006-11. Journal code: 0207224. ISSN: 0006-3363. Pub. country: United States. Language: English.

AB Receptors for GnRH were labeled by use of an iodinated (125I) photoreactive GnRH derivative [D-Lys6-azidobenzoyl]-GnRH. This derivative was found to bind to two classes of GnRH binding sites: high-affinity/low-capacity sites and low-affinity/high-capacity sites. The **binding affinity** of [D-Lys6-azidobenzoyl]-GnRH was found to be greater than that of D-Lys6-GnRH, but lower than a superactive fish GnRH agonist [D-Arg6, Trp7, Leu8, Pro9-NEt]-GnRH (sGnRH-A). Analysis of the photoaffinity-labeled goldfish pituitary GnRH receptors by SDS-PAGE and autoradiography indicated the presence of three labeled proteins displaceable by unlabeled sGnRH-A. The first and the most prominently labeled band was a 71,000-Mr protein, the second a 51,000-Mr protein, and the third a minor band of 130,000 Mr. Displacement characteristics of the 71,000- and 130,000-Mr bands were consistent with those of the low-affinity binding sites; displacement of the iodinated ligand from these proteins was achieved only in the presence of 10(-6) **M** sGnRH-A. The 51,000-Mr band had characteristics similar to those of the high-affinity site; displacement of the labeled ligand was achieved in the presence of 10(-9) **M** sGnRH-A. These findings provide for the first time some biochemical characterizations of pituitary GnRH receptors in a nonmammalian vertebrate.

L7 ANSWER 20 OF 37 MEDLINE on STN

89302082. PubMed ID: 2545195. Photoaffinity labelling of gonadotropin releasing hormone binding sites in human epithelial ovarian carcinomata. Pahwa G S; Vollmer G; Knuppen R; Emons G. (Department of Obstetrics and Gynecology, Medical University of Lubeck, F.R.G.) Biochemical and biophysical research communications, (1989 Jun 30) 161 (3) 1086-92. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB A photoaffinity labelled derivative of [D-Lys6]-GnRH was prepared with a bifunctional photolabile reagent (4-azidobenzoyl)-N-hydroxysuccinimide. In rat pituitary membranes, this analog retained high **binding affinity** (Ka = 0.12 x 10(9) **M**-1) consistent with a single class of receptors. The analog was iodinated and used for the

identification of GnRH binding sites in human epithelial ovarian carcinomata. By sodium dodecyl sulfate electrophoresis in 10% polyacrylamide gel the presence of two labelled components could be demonstrated: a high molecular weight component of 63,200 and a smaller component of 46,000. Competition experiments with unlabelled ligand suggest that it is the high molecular weight component which specifically binds GnRH.

L7 ANSWER 21 OF 37 MEDLINE on STN DUPLICATE 8

89325129. PubMed ID: 2546732. Luteinizing hormone-releasing hormone-binding sites in the rat thymus: characteristics and biological function. Marchetti B; Guarcello V; Morale M C; Bartoloni G; Farinella Z; Cordaro S; Scapagnini U. (Department of Pharmacology, Medical School, University of Catania, Italy.) Endocrinology, (1989 Aug) 125 (2) 1025-36. Journal code: 0375040. ISSN: 0013-7227. Pub. country: United States. Language: English.

AB The present study was designed to explore the effects of **LHRH** and its agonists on immune system function. As a first step, to identify a putative site of action, the very potent and stable **LHRH** agonist (**LHRH**-A), [D-Ser(TBU6)] des-Gly10-**LHRH** ethylamide (buserelin), was used as an iodinated ligand to characterize **LHRH** receptors in a membrane preparation of rat thymus, a key organ of the immune system. The effects of **LHRH** and **LHRH**-A were then investigated on the proliferative capacity of rat thymocytes exposed in vitro to a mitogen and on ornithine decarboxylase specific activity. In addition, to determine whether **LHRH**-A treatment in vivo might directly influence thymic function, we treated hypophysectomized (hypox) rats with a moderately high dose of **LHRH** -A for a period of 2 weeks, and thymocyte mitogenic capacity, thymus weight, and the histological and functional appearance of the thymus were then assessed. Specific binding of **LHRH**-A to rat thymic membrane preparations is a saturable process, depending on both time and temperature of incubation, but differs markedly from binding to the rat pituitary or ovarian **LHRH** receptor in its low **binding affinity.** Binding is optimal in the absence of chelating agents (EDTA) or divalent metal ions, and increases linearly with increasing protein concentration. Binding is specific for **LHRH**, **LHRH**-A, and antagonists. Both the C-terminal amide and N-terminal regions of the **LHRH** molecule were required for binding, and amino acid substitutions at position 6 markedly enhanced and at position 8 markedly reduced binding potencies in rat thymic tissue. A number of peptides, proteins, and other agents had no effect on the specific binding of **LHRH**-A to thymic membrane preparations. The **binding affinity** (Ka) of the membrane receptor of the rat thymus for the **LHRH** superagonist buserelin was 8.4 x 10(8) **M**-1, while a higher **binding affinity** (Ka = 2.8 x 10(9) **M** -1) was calculated for the ovarian **LHRH**-binding site. Preincubation of rat thymocytes with **LHRH**-A for 20 h induced a significant dose-dependent increase in the proliferative response to the mitogen Concanavalin-A, monitored by [3H]thymidine incorporation. Using native **LHRH**, it was also possible to elicit stimulatory effects on the same parameter, although much higher concentrations were required than with **LHRH**-A. Furthermore, simultaneous addition of a **LHRH** antagonist, abolished the **LHRH** effect on thymocytes. Ornithine decarboxylase specific activity under lectin stimulation was also significantly increased by **LHRH**-A in cultures of rat thymocytes.(ABSTRACT TRUNCATED AT 400 WORDS)

L7 ANSWER 22 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN

1988:143775 Document No. 108:143775 Differential sensitivity of agonist- and antagonist-occupied gonadotropin-releasing hormone receptors to protein kinase C activators. A marker for receptor activation. Huckle, William R.; McArdle, Craig A.; Conn, P. Michael (Coll. Med., Univ. Iowa, Iowa City, IA, 52242-1109, USA). Journal of Biological Chemistry, 263(7),

3296-302 (English) 1988. CODEN: JBCHA3. ISSN: 0021-9258.

AB Specific binding of 125I-labeled Buserelin, a high-affinity growth hormone-releasing hormone (GnRH) agonist, was increased to 180% of control in the presence of 150 nM. phorbol 12-myristate 13-acetate (PMA) or 100 nM phorbol 12,13-dibutyrate (PDB), and to 125% of control in the presence of 200 .mu.**M** 1,2-dioctanoylglycerol, after 20 min at 23° in cultured rat pituitary cells. The PMA effects were associated with apparent increases in both **binding affinity** and number of binding sites. The effects of protein kinase C activators on Buserelin binding were concentration- and time-dependent and were not seen with 4α-PMA or 1,2-dioctanoyl-3-chloroglycerol, neither of which activate protein kinase C. In contrast, PMA had no measurable effects on specific binding of a GnRH receptor antagonist. When cell cultures were pretreated with 100 nM PDB in the absence of GnRH and then washed to remove the phorbol ester, no effects of prior protein kinase C activation were detected after subsequent addition of Buserelin. However, when PDB pretreatment was carried out in the presence of 0.3 .mu.**M** GnRH, residual enhancement of Buserelin binding, but not antagonist binding, was observed at either 23 or 4°. The radiolabeled agonist activated, and the antagonist blocked, GnRH receptor-mediated LH release and [3H]inositol phosphate production in cells preloaded with [3H]inositol. Thus, the action of protein kinase C on the GnRH receptor, either direct or indirect, requires the receptor to be in an activated (agonist-occupied) state but does not require receptor internalization. The mechanism of these effects on GnRH agonist binding is not known but may involve sequestration of surface receptors, expression of new receptors, and(or) modulation of GnRH receptor affinity.

L7 ANSWER 23 OF 37 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN DUPLICATE 9

1988:180340 Document No.: PREV198885092442; BA85:92442. RECEPTORS FOR PROLACTIN SOMATOSTATIN AND **LHRH** IN EXPERIMENTAL PROSTATE CANCER AFTER TREATMENT WITH ANALOGS OF **LHRH** AND SOMATOSTATIN. KADAR T [Reprint author]; REDDING T W; BEN-DAVID M; SCHALLY A V. ENDOCRINE, POLYPEPTIDE CANCER INST, TULANE UNIV SCH MED, NEW ORLEANS, LA 70146, USA. Proceedings of the National Academy of Sciences of the United States of America, (1988) Vol. 85, No. 3, pp. 890-894.
CODEN: PNASA6. ISSN: 0027-8424. Language: ENGLISH.

AB Membrane receptors for luteinizing hormone-releasing hormone (LH-RH) somatostatin, and prolactin(PRL) were investigated in the Dunning R-3327H rat prostate adenocarcinoma specimens after in vivo treatment with microcapsules of the agonist [D-Trp6]LH-RH and the somatostatin analog RC-160. The LH=RH receptors showed a low-**binding affinity** (Kd = 54 nM) and high capacity (Bmax = 12.0 pmol/mg). Treatment with the [D-Trp6]LH-RH decreased the **binding affinity** (Kd = 0.52 .mu.**M**). Specific somatostatin receptors, with Kd = 1.3 nM and Bmax = 543 fmol/mg, were also found. Treatment with [D-Trp6]LH-RH lowered Bmax to 44 fmol/mg, and administration of RC-160 reduced Kd to 30 nM. After the combined treatment with the two analogs, Kd and Bmax were decreased. Specific PRL receptors (Kd = 0.72 nM; Bmax = 161 fmol/mg) were also detected. Treatment with either analog reduced Bmax by 50%, but a much greater reduction of PRL binding capacity was revealed after in vitro dissociation of the bound endogenous PRL by MgCl2. The dramatic fall in the total number of PRL receptors after combination treatment with both analogs could be partially responsible for the decrease in the weight and volume of prostate tumors. The findings support the concept that analogs of LH-RH and somatostatin can inhibit tumors directly through their own respective receptors. One of several mechanisms of the antineoplastic activity of these analogs could be the elimination of tumor growth-promoting effect of PRL by the reduction of the total number of PRL receptors.

L7 ANSWER 24 OF 37 MEDLINE on STN

89050085. PubMed ID: 2847713. Fibroblast growth factor regulates the expression of luteinizing hormone receptors in cultured rat granulosa cells. Oury F; Darbon J M. (Inserm U 168, Department of Endocrinology, CHU Rangueil, Universite Paul Sabatier, Toulouse, France.) Biochemical and biophysical research communications, (1988 Oct 31) 156 (2) 634-43. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB We have investigated the effects of bFGF on both the FSH-induced LH receptor expression and cAMP production in cultured rat granulosa cells. Concentrations of pure FGF, from 10(-12) **M** to 10(-10) **M**, progressively inhibit the stimulatory actions of FSH with an ED50 of approximately 4 x 10(-12) **M** for both parameters. Higher FGF concentrations, from 4 x 10(-10) **M** to 10(-8) **M**, lead to a gradual reduction of the growth factor inhibitory effect. The effects of FGF are more prominent on the modulation of LH receptors than on the FSH-induced cAMP production. Moreover, FGF impairs the LH receptor formation induced by cholera toxin or 8-Bromo-cAMP, indicating that the growth factor also acts at a step distal to cAMP formation. The inhibitory effect of FGF on LH receptor expression increases during the entire course of granulosa cell differentiation, from 24 to 96 h, and is not due to variations in cell number or viability, but rather to a change in the content of LH receptors with no significant modification of **binding affinity** (KD congruent to 0.8 x 10(-10) **M**). These results suggest that bFGF may acutely regulate the capacity of granulosa cells to differentiate upon FSH stimulation and to respond to LH during the ovarian follicular maturation.

L7 ANSWER 25 OF 37 MEDLINE on STN DUPLICATE 10

89351546. PubMed ID: 2855595. Solubilization and characterization of the rat pituitary gonadotrophin-releasing hormone receptor. Iwashita M; Hirota K; Izumi S I; Chen H C; Catt K J. (Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, Bethesda, Maryland 20892.) Journal of molecular endocrinology, (1988 Nov) 1 (3) 187-96. Journal code: 8902617. ISSN: 0952-5041. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Specific receptors for gonadotrophin-releasing hormone (GnRH) were extracted from the rat pituitary gland with several detergents and characterized by binding studies with the potent GnRH antagonist [Ac-D-pCl-Phe1.2, D-Trp3, D-Lys6, D-Ala10]-GnRH (GnRHant). The particulate GnRH receptors were most effectively solubilized with 5 mM 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate (CHAPS), which extracted 63% of the original membrane binding activity when assayed with 125I-labelled GnRHant. The **binding affinities** of particulate and CHAPS-solubilized receptors analysed with 125I-labelled GnRHant were 1.5 +/- 0.4 x 10(9) **M**-1 and 1.2 +/- 0.2 x 10(9) **M**-1 respectively. Gel filtration of the CHAPS-solubilized receptor revealed a major peak of specific binding activity with Mr of about 700,000. A hormone-receptor complex of similar Mr was observed when CHAPS-solubilized receptors were labelled with photoreactive radioiodinated [D-Lys6]-des-Gly10-GnRH-N-ethyl-amide and then analysed by gel chromatography. However, when pituitary particles were photolabelled and solubilized in 2% Triton X-100 before analysis on Sephacryl S-300, the Mr of the receptor was approximately 250,000, similar to the value obtained by sucrose density gradient centrifugation of the CHAPS-solubilized receptor. After solubilization in sodium dodecyl sulphate (SDS) the photolabelled receptor was eluted from Sephacryl S-300 as a 60 kDa peak which on SDS-gel electrophoresis contained a 52 kDa component, corresponding to the major binding subunit extracted directly from photolabelled pituitary membranes. The difference in higher molecular weight forms observed under non-denaturing and denaturing conditions could reflect the need for additional membrane components to maintain the active conformation of the GnRH receptor site. Whereas the minimum Mr of the solubilized receptor is about 250,000 under non-denaturing conditions, analysis of the photolabelled GnRH-receptor

complex by SDS chromatography and electrophoresis indicates that a binding subunit with Mr of 50,000-60,000 is present in the GnRH holoreceptor.

L7 ANSWER 26 OF 37 MEDLINE on STN DUPLICATE 11
88294670. PubMed ID: 2841008. Distribution of gonadrotropin releasing hormone agonist binding sites in the rat central nervous system. Jennes L; Dalati B; Conn P M. (Department of Anatomy, Wright State University, Dayton, OH 45435.) Brain research, (1988 Jun 14) 452 (1-2) 156-64. Journal code: 0045503. ISSN: 0006-8993. Pub. country: Netherlands. Language: English.

AB Specific binding sites for gonadotropin releasing hormone (GnRH) in the central nervous system of the rat were studied with in vitro autoradiography and with radioligand assays. The results show that GnRH binding sites are present in the lamina glomerulosa and plexiformis externa, the nucleus olfactorius anterior pars externa, and the frontal cortex at the sulcus rhinalis. In the septum, only a few GnRH binding sites are detected in the lateral and dorsal portions of the nucleus septi lateralis. In addition, a small number of GnRH receptors are seen in the mediobasal hypothalamus and amygdala while substantial binding is apparent in the interpeduncular nucleus, central gray and superior collicle. In the hippocampal formation the GnRH agonists bind to the dorsal and ventral subiculum as well as to receptors in the areas CA1 through CA4. The highest concentration of GnRH receptors is found in the parasubiculum. Competitive binding assays with membrane preparations from the hippocampus and interpeduncular nucleus indicate that the binding of the GnRH agonists is reversible and has a **binding affinity** of 1 X 10(9) **M**-1. Injections of radioactive GnRH agonist Buserelin into the lateral ventricle results in selective and reversible labeling of the hippocampal areas CA1 through CA4 as well as the interpeduncular nucleus, central gray and the parasubiculum. The results of the present study indicate that GnRH binds to specific receptors in select areas of the central nervous system of the rat where the peptide may regulate sensory, behavioral and endocrine events. (ABSTRACT TRUNCATED AT 250 WORDS)

L7 ANSWER 27 OF 37 MEDLINE on STN DUPLICATE 12
87051079. PubMed ID: 3536044. On the role of luteinizing hormone-releasing hormone in the in vitro synthesis of bioactive human chorionic gonadotropin in human pregnancies. Belisle S; Bellabarba D; Gallo-Payet N; Lehoux J G; Guevin J F. Canadian journal of physiology and pharmacology, (1986 Sep) 64 (9) 1229-35. Journal code: 0372712. ISSN: 0008-4212. Pub. country: Canada. Language: English.

AB The dynamics of luteinizing hormone-releasing hormone (**LHRH**) induced human chorionic gonadotropin (hCG) production were studied in isolated placental cells from normal and anencephalic midterm and term gestations. A spontaneous release of immunoreactive hCG was first detected after 24-36 h of preparation in term control cells. The addition of **LHRH** at a concentration ranging from 10(-9) to 10(-6) **M** induced a threefold increase in this output of hCG. Placental cell responsiveness to **LHRH** varied according to the number of days of cell cultures, with maximal response on days 1 and 6. Placental cells from normal pregnancies incubated with 1 X 10(-6) **M** **LHRH** showed a release of both immuno- and bio-assayable hCG, which was four- to six-fold higher at midgestation than at term (p less than 0.001). In contrast, placental cells from pregnancies with anencephalic fetuses showed, at both stages of gestation, an hCG production that was comparable to that observed with normal term placental cells. We conclude that **LHRH** at a concentration appropriate for its placental receptor **binding affinity** induces a production of bioactive hCG in humans. Furthermore, our data suggest that anencephaly changes the placental response of hCG to **LHRH** stimulation.

L7 ANSWER 28 OF 37 MEDLINE on STN
86059935. PubMed ID: 2999176. Characterization of a gonadotropin-releasing hormone receptor site in term placenta and chorionic villi. Iwashita M;

Evans M I; Catt K J. Journal of clinical endocrinology and metabolism, (1986 Jan) 62 (1) 127-33. Journal code: 0375362. ISSN: 0021-972X. Pub. country: United States. Language: English.

AB The properties of GnRH receptor sites in the human placenta were analyzed by binding studies performed in particulate and solubilized receptor preparations. The **binding affinities** (Ka) of the membrane receptor of term placenta for the GnRH superagonists [D-Lys6]- and [D-Ala6]des-Gly10- GnRH-N-ethylamide were 1.6 X 10(6) and 5.4 X 10(5) **M**-1, respectively. The **binding affinities** of native GnRH and a potent GnRH antagonist were similar to those of the superagonists (1.1 and 2.0 X 10(6) **M**-1, respectively). The placental sites could be solubilized by extraction with the detergent 3-[(3-cholamidopropyl) dimethylammonio] 1-propane sulfonate, with retention of 40-45% of the specific binding activity, though with a significant decrease in **binding affinity.** The sites were also solubilized with sodium dodecyl sulfate after covalent labeling with a photoreactive 125I-labeled [D-Lys6] GnRH agonist derivative. Analysis of the solubilized hormone-receptor complex on polyacrylamide gradient gels showed a single band of 53,700 mol wt, similar to the mol wt of the pituitary GnRH receptor in other species. It is clear that the placental receptor differs markedly from the GnRH receptor of the pituitary gland in its low **binding affinity** and lack of selectivity for GnRH analogs. However, it is possible that the human placental receptor for GnRH could serve as a low affinity regulatory site for locally formed GnRH or related low affinity regulatory site for locally formed GnRH or related peptides within the placenta, and that the placental GnRH system has a significant role in the maintenance of pregnancy.

L7 ANSWER 29 OF 37 MEDLINE on STN

85182338. PubMed ID: 2985529. **LHRH** receptor, LH and FSH concentrations in anterior pituitaries of cycling, noncycling and early pregnant heifers. Schoenemann H M; Brown J L; Reeves J J. Journal of animal science, (1985 Feb) 60 (2) 532-6. Journal code: 8003002. ISSN: 0021-8812. Pub. country: United States. Language: English.

AB In domestic animals limited data are available concerning levels of pituitary luteinizing hormone-releasing hormone (**LHRH**) receptors during various physiological states. The objectives of this study were to quantify anterior pituitary gonadotropin and **LHRH** receptor concentrations in cycling, noncycling and early pregnant beef heifers. To accomplish these objectives, five heifers each were slaughtered, after synchronization with prostaglandin F2 alpha (PGF2 alpha), on d 0 (estrus), 7 and 14 of the estrous cycle and d 40 of pregnancy. Four heifers determined to be noncycling were also slaughtered. Pituitaries were collected and analyzed for **LHRH** receptor and gonadotropin concentrations. Pituitary luteinizing hormone (LH) concentrations were low on d 0 (1.4 +/- .2 micrograms/mg pituitary, mean +/- SE) and remained low on d 7 (1.4 +/- .1 micrograms/mg pituitary) before increasing (P less than .01) on d 14 (2.6 +/- .5 micrograms/mg pituitary). Luteinizing hormone concentrations, compared with d 0, were also elevated (P less than .01) in noncycling (NC; 2.6 +/- .2 micrograms/mg pituitary) animals and in 40-d pregnant (PG; 2.5 +/- .2 micrograms/mg pituitary) heifers. Pituitary follicle stimulating hormone (FSH) concentrations, though similar (P greater than .05) for all groups, paralleled changes in LH concentration. Pituitary **LHRH** receptor **binding affinity** did not differ (P greater than .05) among groups, with an overall Kd = .64 +/- .02 X 10(-9) **M.** Luteinizing hormone-releasing hormone receptor concentrations were highest on d 0 (1.09 +/- .12 fmol/mg pituitary) and fell (P less than .01) to low levels on d 7 (.75 +/- .11 fmol/mg pituitary).(ABSTRACT TRUNCATED AT 250 WORDS)

L7 ANSWER 30 OF 37 MEDLINE on STN

84135781. PubMed ID: 6321484. Receptors and inhibitory actions of gonadotropin-releasing hormone in the fetal Leydig cell. Dufau M L; Warren

D W; Knox G F; Loumaye E; Castellon M L; Luna S; Catt K J. Journal of biological chemistry, (1984 Mar 10) 259 (5) 2896-9. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The receptors and actions of gonadotropin-releasing hormone (GnRH) were analyzed in cultured testicular cells from 20.5-day fetal rats, in which treatment with luteinizing hormone (LH) maintained Leydig cell steroidogenesis and gonadotropic responses for up to 2 weeks. Testicular GnRH receptors were present on the 5th postnatal day, but were not demonstrable in fetal testes or 2-day cultures thereof. However, GnRH receptors were readily detectable in 4-day cultured fetal testes and were increased by exposure to GnRH agonists. In LH-treated cultures, GnRH sites were reduced by about 50% and did not increase during incubation with GnRH agonists. In such cultures, GnRH agonists inhibited LH-dependent steroid production and abolished the acute testosterone response to human chorionic gonadotropin. The half-maximal inhibitory concentration of [D-Ala6]des-Gly10-GnRH-N-ethylamide (3 X 10(-10)**M**) was commensurate with its **binding affinity** for testis receptors (Kd = 1.4 X 10(-10)**M**). In contrast, GnRH agonists had no inhibitory effects in 2-day cultures prior to the detection of GnRH receptors. The expression of functional GnRH receptors during culture in the absence of gonadotropin and their suppression in LH-treated cultures suggest that pituitary gonadotropins exert a tonic inhibitory effect upon testicular GnRH receptors. The demonstrated inhibitory actions of GnRH on steroidogenesis, with the expression of GnRH receptors in cultured fetal testes and 5-day postnatal testes, indicate that GnRH agonists could influence the actions of gonadotropins upon Leydig cell function in the neonatal testis.

L7 ANSWER 31 OF 37 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

1985:272751 Document No.: PREV198579052747; BA79:52747. IDENTIFICATION AND CHARACTERIZATION OF **LHRH** RECEPTORS IN THE ENDOMETRIAL TISSUE OF PREGNANT RATS. CAO Y [Reprint author]; SHEN X; YE L; CHEN Y; ZHANG Z. DEP ENDOCRINOL, INST ZOOL, ACAD SINICA, BEIJING, CHINA. Scientia Sinica Series B (Chemical Biological Agricultural Medical and Earth Sciences), (1984) Vol. 27, No. 7, pp. 687-695.
CODEN: SSBSEF. ISSN: 0253-5823. Language: ENGLISH.

AB The mechanisms by which **LHRH** or its agonists inhibit decidua formation and terminate pregnancy in rats are not clear. If the gonadotropic releasing hormone or its analogs act directly on the uterus, then it should be possible to identify **LHRH** receptors in the endometrial tissue. The homogenate preparation of rat endometrial tissue bound 125I-**LHRH** with an affinity constant of Ka = 1.3 + 108 **M**-1. The binding capacity was measured as 236 fmol/mg protein. The binding is highly specific, because no binding was observed in a variety of non-target tissues, such as liver, kidney and muscle. Competitive binding with TRH, substance P and bradykinin did not occur under experimental conditions. Day 3 of pregnancy was a critical time showing a higher binding activity. [The antifertility and abortifacient mechanisms are examined.].

L7 ANSWER 32 OF 37 MEDLINE on STN DUPLICATE 13

84265179. PubMed ID: 6378971. High affinity monoclonal antibodies to luteinizing hormone-releasing hormone. Preparation and binding studies. Knapp R J; Sternberger L A. Journal of neuroimmunology, (1984 Aug) 6 (5) 361-71. Journal code: 8109498. ISSN: 0165-5728. Pub. country: Netherlands. Language: English.

AB Two mouse hybridoma cell lines (50-1 and 50-11) secreting high affinity, monoclonal IgG antibodies (MCAs) against luteinizing hormone-releasing hormone (**LHRH**) have been established. Measurements of **binding affinity** by both equilibrium and kinetic methods give dissociation constants (Kd) of 2.3-3.2 X 10(-10) **M** for 50-1 and 3.0 X 10(-10) **M** for 50-11. Analysis of binding specificity show that both MCAs require the C-terminal glycine amide and adjacent 4

amino acids of **LHRH** for binding. The use of these MCAs in radioimmunoassay (RIA) is described.

L7 ANSWER 33 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
1984:45415 Document No. 100:45415 Localization of biotinylated gonadotropin releasing hormone on pituitary monolayer cells with avidin-biotin-peroxidase complexes. Childs, Gwen V.; Naor, Zvi; Hazum, Eli; Tibolt, Robert; Westlund, Karin N.; Hancock, Michael B. (Med. Branch, Univ. Texas, Galveston, TX, USA). Journal of Histochemistry and Cytochemistry, 31(12), 1422-5 (English) 1983. CODEN: JHCYAS. ISSN: 0022-1554.

AB The avidin-biotin-peroxidase complex (ABC) technique was used to localize the [D-Lys6] analog of gonadotropin releasing hormone (GnRH) [9034-40-6], labeled with biotin, on pituitary monolayer cultures from female rats. Staining was diffuse, or in patches, on the surface of 10-17% of the cells 30 sec-3 min after the addition of 10-10-10-12 **M** biotin-labeled GnRH. In parallel studies, double stains for gonadotropins showed label on 16.3% of the monolayers. Capping was evident by 3 min after exposure and the stain appeared in dense patches, vesicles, or granules 10-30 min after exposure. The stain was abolished by the addition of a 10-100-fold excess of unlabeled [D-Lys6] GnRH. Biotinylated GnRH released LH [9002-67-9] and FSH [9002-68-0] and was either equipotent or 10 times more potent than the unlabeled analog in multiple dose-response tests. The ED50 of the 4 h release was 0.075 nM for LH and 0.02 nM for FSH. Competitive binding assays showed that the **binding affinity** of the biotinylated GnRH was within the range found for the unlabeled analog (0.7 nM-IC50). Thus, the localization of biotinylated GnRH on the surfaces of cells exposed to low concns. of the analog was determined with a technique that requires minimal manipulation of the cells and is performed in <1 day.

L7 ANSWER 34 OF 37 MEDLINE on STN DUPLICATE 14
82232305. PubMed ID: 6807361. Analysis of androgen action on pituitary gonadotropin and prolactin secretion in ewes. Clarke I J; Mitchelhill K; Zachariah E; Findlay J K; Funder J W. Biology of reproduction, (1982 Jun) 26 (5) 777-86. Journal code: 0207224. ISSN: 0006-3363. Pub. country: United States. Language: English.

AB To study the role of androgens in the control of gonadotropin and prolactin secretion in ther ewe, we have characterized androgen receptors in pituitary cytosol, and investigated the effect of androgens on pituitary hormone release in vivo and in vitro. High affinity, low capacity receptors, with an affinity for methyltrienolone (R1881) greater than 5 alpha-dihydrotestosterone (5 alpha-DHT) greater than testosterone (T) much greater than androstenedione (A4), estradiol-17 beta (E2) and progesterone (P), were identified in pituitary cytosol. Addition of 1 nM 5 alpha-DHT, but not A4, inhibited luteinizing hormone (LH) release from pituitary cells in vitro, induced by 10(10) to 10(-7) **M** luteinizing hormone releasing hormone (**LHRH**). The release of follicle-stimulating hormone (FSH) with 10(-9) **M LHRH** was inhibited when cells were incubated with 1 nM 5 alpha-DHT. 5 alpha-DHT had no effect when higher or lower doses of **LHRH** were used. In ovariectomized ewes, neither an i.v. injection of 1 mg, nor intracarotid injections of up to 1 mg, 5 alpha-DHT affected plasma LH, FSH or prolactin levels, despite dose-related increases in plasma 5 alpha-DHT levels. Daily or twice daily i.**m.** injections of 5 mg 5 alpha-DHT in oil did not affect LH or FSH levels, but daily injections of 20 mg significantly reduced plasma LH levels within 4 days and plasma FSH levels within 6 days. Thus, despite the presence of androgen receptors in the ewe pituitary, we conclude that androgens per se are of minimal importance in the regulation of pituitary LH, FSH and prolactin secretion in the ewe. The low **binding affinity** of A4 and the lack of its effect on hormone secretion in vitro suggests that A4 may act as an estrogen precursor rather than an androgenic hormone. The function of the pituitary androgen receptor remains to be established.

L7 ANSWER 35 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
1980:421985 Document No. 93:21985 Radioligand assay for gonadotropin-releasing hormone: relative potencies of agonists and antagonists. Perrin, Marilyn H.; Rivier, Jean E.; Vale, Wylie W. (Peptide Biol. Lab., Salk Inst. Biol. Stud., La Jolla, CA, 92037, USA). Endocrinology, 106(4), 1289-96 (English) 1980. CODEN: ENDOAO. ISSN: 0013-7227.

AB A radioligand assay employing tritiated gonadotropin-releasing hormone (GnRH), GnRH-9-prOline-3H or GnRH-1-pyroglutamate-3H, was used to investigate the binding of GnRH, its agonists, and its antagonists to male rat anterior pituitary homogenates. The tritiated GnRH purified by high pressure liquid chromatog. and stored in 10 mM HOAc is stable for binding for at least 14 wk. There was at least 1 high affinity site with an observed Kd of .simeq.2 nM and another low affinity site whose Kd was .simeq.1 μ **M.** Only .apprx.25% of the total specific binding was due to the low affinity site. At room temperature, the binding was reduced to 50% of that at 0°, and at 37°, there was no measurable binding. Bacitracin had no effect on the binding at any temperature Maximum binding occurred between pH 7.5-8.5. The quant. relative binding potencies of several agonists and antagonists were determined These potencies closely paralleled their biol. potencies, but all antagonists had higher absolute **binding affinities** when compared to their potencies to inhibit GnRH-mediated LH secretion in vitro.

L7 ANSWER 36 OF 37 CAPLUS COPYRIGHT 2005 ACS on STN
1980:579913 Document No. 93:179913 Ovarian gonadotropin-releasing hormone receptors. I. Properties and inhibition of luteal cell function. Harwood, James P.; Clayton, Richard N.; Catt, Kevin J. (Endocrinol. Reprod. Res. Branch, Natl. Inst. Child Health Hum. Dev., Bethesda, MD, 20205, USA). Endocrinology, 107(2), 407-13 (English) 1980. CODEN: ENDOAO. ISSN: 0013-7227.

AB In collagenase-dispersed rat luteal cells, the gonadotropin-releasing hormone (GnRH) [9034-40-6] agonist analog [D-Ser(tBu)6]des-Gly10-GnRH N-ethylamide (GnRHa) caused inhibition of human chorionic gonadotropin (hCG) [9002-61-3]-stimulated progesterone [57-83-0] production Epinephrine [51-43-4]-stimulated progesterone production in isolated luteal cells was also partially inhibited by GnRH. On the other hand, there was no effect of the analog on the steroid dose-response curve to dibutyryl cAMP [362-74-3]. The effect of GnRHa on the steroidogenic response to both hormones was to shift the dose-dependent curve to the right without changing the maximum production of progesterone. GnRH also inhibited steroid production with a half-maximal effect occurring at 5 + 10-9M in agreement with the **binding affinity** of GnRH for its gonadal receptors (Ka = 5 + 108 **M**-1). GnRHa also caused a shift to the right in the dose-dependent curve for cAMP [60-92-4] production stimulated by hCG. However, no direct inhibitory effects of GnRH and its agonist analogs was detected on hCG- or epinephrine-stimulated adenylate cyclase [9012-42-4] activity measured on ovarian homogenates. GnRH did not affect the **binding affinity** or capacity of rat ovarian receptors for hCG. Specific GnRH receptors in luteinized rat ovaries were identified with 125I-labeled GnRHa and found to have high affinities for the analog (Ka = 5 + 109M-1). A potent antagonist analog ([D-pyro-Glu1, D-Phe2,D-Trp3,6]GnRH [68059-94-9]) was also bound with high affinity (Ka = 3 + 109M-1) by the ovarian receptors. GTP, guanosine 5'-(β-γ-imido)triphosphate, and ATP as well as prostaglandin F2, and epinephrine did not alter binding of the analog to GnRH receptors. The ovary contains specific, high affinity receptors for GnRH, through which GnRH and its agonist analogs can inhibit steroidogenic responses to hormonal stimulation. GnRH apparently interferes with the mechanism of hormonal stimulation of cAMP production

L7 ANSWER 37 OF 37 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 15
80216496 EMBASE Document No.: 1980216496. [Pituitary **LHRH** receptor sites: Quantification with a superactive analog as tracer,

structure-function relationship, and change during castration in male rats]. RECEPTEURS **LHRH** DANS L'HYPOPHYSE: UTILISATION D'UN ANALOGUE SUPERACTIF COMME TRACEUR POUR LEUR DETECTION; RELATION STRUCTURE-ACTIVITE; EVOLUTION DE CES RECEPTEURS APRES CASTRATION CHEZ LE RAT MALE. Aubert M.L.; Conne B.S.; Scaglioni S.; Sizonenko P.C.. Unite Endocrinol., Dept. Ped. Genet., Fac. Med., Univ. Geneve, CH-1211 Geneve 4, Switzerland. Journal de Physiologie Vol. 76, No. 3, pp. 207-218 1980. CODEN: JOPHAN
Pub. Country: France. Language: French. Summary Language: English.

ED Entered STN: 911209

AB Bnding sites or 'receptors' for **LHRH** (Luteinizing Hormone-Releasing Hormone) can be detected in the pituitary gland of rats, sheep or cattle. These sites can be quantified by the use of tritiated or radioiodinated native **LHRH**, but the total binding obtained is usually low (1-8%) and the tracer is easily damaged during incubation. The use as tracer of the highly potent analog DesGly10[DTrp6-(N-Et)Pro9]-**LHRH**, which possessed 144 times the biological activity of native **LHRH** and is resistant to brain proteolytic enzymes, allows a very precise measurement of these pituitary sites. This **LHRH** analog, which can be easily radioiodinated by the lacto-peroxidase-glucose-oxidase method, binds very acidly to preparations of pituitary membranes. Up to 60% of the tracer can be bound. Scatchard analysis of saturation curves indicates only one class of high affinity (K(A) = 1010 **M**-1) and low affinity binding sites. Complete displacement of bound radioiodinated **LHRH** analog from pituitary binding sites is achieved not only by the analog itself, but also by native **LHRH** and by many different analogs or antagonists of **LHRH.** Receptor affinity of **LHRH** analogs was proportional to biological activity. **Binding affinity** of **LHRH** antagonists was proportional to antagonistic activity. For example, DpGlu1-DPhe(2,6)-DTrp3-**LHRH**, which is able to recognize native **LHRH** at a **molar** ratio of 5 : 1, exhibited a **binding affinity** for pituitary receptors 3.5 times that of native **LHRH.** Castration of 60 day old rats produced a rapid and sustained increase of **LHRH** receptor sites, in parallel to the well known increase of plasma gonadotrophin secretion. Forty eight hours after castration, **LHRH** receptor concentration was 446 fmole/mg (plasma LH = 231 ± 15 ng/ml) in castrates, 68 (37 ± 3) in sham-operated animals, and 181 (30 ± 3) in controls. Thirteen days after castration, **LHRH** receptor concentration was 478 (318 ± 3) in castrates, 166 (34 ± 3) in sham-operated animals, and 208 (38 ± 3) in controls. **LHRH** content in the hypothalamus decreased markedly after castration (0.89 ± 0.29 ng after 2 days, and 0.61 ± 0.19 ng after 13 days) as compared to sham-operated animals (3.99 ± 0.3 and after 2 days, and 2.28 ± 0.26 ng after 13 days). In conclusion, the use of a very active analog of **LHRH** as tracer allows an easy and reliable measurement of pituitary **LHRH** binding sites which are likely to represent specific receptor sites. Knowledge of pituitary **LHRH** receptor concentration represents a novel approach for a better understanding of the mechanisms involved in the regulation of gonadotrophin secretion.

```
=> s l1 and "10-4M"
L10          10 L1 AND "10-4M"

=> dup remove l10
PROCESSING COMPLETED FOR L10
L11           9 DUP REMOVE L10 (1 DUPLICATE REMOVED)

=> d l11 1-9 cbib abs
```

L11 ANSWER 1 OF 9 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED.
on STN

2000187643 EMBASE Effects of ascorbic acid on hepatic nuclear binding of L-tryptophan. Sidransky H.; Verney E.. Dr. H. Sidransky, Department of Pathology, George Washington Univ. Med. Center, Washington, DC 20037, United States. Nutrition Research Vol. 20, No. 6, pp. 865-875 2000.
Refs: 18.
ISSN: 0271-5317. CODEN: NTRSDC
S 0271-5317(00)00168-8. Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 20000622
AB This study investigated the in vitro effects of ascorbic acid on nuclear L-tryptophan receptor binding of rat liver. Ascorbic acid at 10-6M to **10- 4M** inhibited the in vitro 3H-tryptophan binding to hepatic nuclei and nuclear envelope receptors. Addition of dithiothreitol, a protective agent for sulfhydryl groups, did not affect the inhibitory binding. Addition of L- leucine did reduce the inhibitory binding. Addition of NaCl (0.125 x 10-4 **M**) did not influence the inhibitory binding effect of ascorbic acid but decreased the inhibitory effect of the NaCl on nuclear tryptophan binding. In vivo administration of ascorbic acid before or along with L-tryptophan decreased the L-tryptophan-induced stimulation of hepatic protein synthesis. (C) 2000 Elsevier Science Inc.

L11 ANSWER 2 OF 9 EMBASE
on STN
95059780 EMBASE Document No.: 1995059780. A comparative study of the biochemical properties of human and mouse recombinant O6-methylguanine-DNA methyltransferases. Roy R.; Shiota S.; Kennel S.J.; Raha R.; Von Wronski M.; Brent T.P.; Mitra S.. Department of Microbiology, School of Dentistry, Kyushu University,Fukuoka, Japan. Carcinogenesis Vol. 16, No. 2, pp. 405-411 1995.
ISSN: 0143-3334. CODEN: CRNGDP
Pub. Country: United Kingdom. Language: English. Summary Language: English.
ED Entered STN: 950308
AB The O6-methylguanine-DNA methyltransferase (MGMT) repairs mutagenic and carcinogenic O6-alkylguanine in DNA by accepting stoichiometrically the alkyl group from the base. Although the mouse MGMT is larger than the human protein because of an additional tetrapeptide sequence, these proteins are 70% homologous. Recombinant MGMTs of the human, the mouse and a mouse mutant with the tetrapeptide deleted were purified to homogeneity from Escherichia coli. The N-terminal amino acid sequences of these proteins are identical to those predicted from the nucleotide sequences, and their molecular masses determined by SDS-PAGE agreed with the predicted values. However, the observed isoelectric points of 9.3, 9.2 and 9.3, for the human, mouse and mutant mouse proteins respectively were significantly different from the values, 8.09, 7.47 and 7.49 calculated from the amino acid composition. The extinction coefficients E(1%)(280 nm) of human, mouse and mutant mouse protein were calculated from amino acid composition to be 18.2, 11.1 and 11.3 respectively. These values agree fairly well with calculated values. Human and wild-type mouse MGMTs react with the alkylated base in a synthetic DNA substrate poly(dC, dG, m6dG) with comparable second-order rate constants of 2.2 x 108 and 3.7 x 108 l/**M**/min at 37°C respectively and were inactivated by O6-benzylguanine at similar rates. The initial reaction rate (K(in)) and rate of inactivation (k(inact)) constants for reaction with the base were calculated to be 1.8 x 10-4 **M** and 1.4 x 10-3/s for the human protein, 2.3 x 10-4 **M** and 1.1 x 10-3/s for the wild-type mouse protein, and 2.1 x **10-4M** and 1.4 x 10-3/s for the mutant mouse protein respectively. The MGMTs were inactivated to the extent of 55-65% after heating at 50°C in 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1 mM DTT and 10% glycerol. However, in the presence of DNA (200 μg/ml), only 25-35% of the protein was inactivated. Both DNA and RNA inhibited all three enzymes in a concentration-dependent fashion, although DNA was a better inhibitor than

RNA. High salt (0.2 **M** NaCl) inhibited human MGMT by 80%, while the wild-type and the mutant mouse MGMTs were inhibited by 55%. The human protein had higher affinity for binding to duplex DNAs than the mouse proteins. Immunoprecipitation (69%) and affinity constant (19.4 nM) of human MGMT with a human-specific monoclonal antibody 4.A1 significantly discriminated the human protein from either of the mouse proteins.

L11 ANSWER 3 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
1994:530316 Document No. 121:130316 β-Adrenoreceptors in the trout (Oncorhynchus mykiss) heart: characterization, quantification, and effects of repeated catecholamine exposure. Gamperl, A. K.; Wilkinson, M.; Boutilier, R. G. (Dep. Biol., Dalhousie Univ., Halifax, NS, B3H 4J1, Can.). General and Comparative Endocrinology, 95, 259-72 (English) 1994. CODEN: GCENA5. ISSN: 0016-6480.

AB Specific binding of the hydrophilic radioligand [3H]CGP-12177 to cell surface (functional) β-adrenoreceptors was quantified in ventricular micropunches (2-mm diameter, 350-.mu.**m** thickness) from seawater-acclimated rainbow trout held at 7-9°. Binding was stereospecific, saturable, of high affinity, and displaceable by appropriate agonists and antagonists. Phentolamine failed to displace [3H]CGP at <**10-4M**, indicating an absence of [3H]CGP binding to α-adrenergic receptors. Trout ventricular β-adrenoreceptors are exclusively of the β2 type. This conclusion is based on: (1) the IC50 value for the β2-antagonist ICI 118551 (2.9 + 10-6M); (2) the inability of the β1-antagonist atenolol to displace [3H]CGP from β-adrenoreceptors; and (3) the order of agonist-**binding affinity** (isoproterenol > epinephrine » norepinephrine). The βmax and Kd values for [3H]CGP binding to myocardial tissue were .apprx.0.04 fmol/μg protein and 0.25 nM, resp. The Bmax value indicates that the d. of cell surface (functional) β-adrenoreceptors in the ventricle was 12,000 sites per cell or 3.38 sites per μm2 of sarcolemma. The Kd and Bmax values for [3H]CGP binding to ventricular β-adrenoreceptors were unaffected by the in vivo administration of 5 bolus catecholamine injections (4.0 μg/kg epinephrine, 2.0 μg/kg norepinephrine). This suggests that stress-induced increases in plasma catecholamines are unlikely to cause the down-regulation of heart β-adrenoreceptors in fish. The method described here represents a simple but powerful technique for the quantification and characterization of adrenergic receptors in the fish heart.

L11 ANSWER 4 OF 9 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
94177934 EMBASE Document No.: 1994177934. Free DNA concentration in E. coli estimated by an analysis of competition for DNA binding proteins. Stickle D.F.; Vossen M.; Riley D.A.; Fried M.G.. Department of Biological Chemistry, Pennsylvania State Univ.College Med.,Hershey, PA 17033, United States. Journal of Theoretical Biology Vol. 168, No. 1, pp. 1-12 1994. ISSN: 0022-5193. CODEN: JTBIAP
Pub. Country: United Kingdom. Language: English. Summary Language: English.

ED Entered STN: 940720

AB Transcription in E. coli is often controlled by the binding of specific gene-regulatory proteins. Binding of these proteins to their specific DNA binding sites occurs in the presence of a large excess of 'nonspecific' genomic DNA. Binding to a specific DNA site thus depends on the concentration of regulatory protein, on its affinities for specific and competing nonspecific binding sites, and on the free concentrations of those sites. Although it is probable that genomic DNA is largely occluded by protein binding or by condensation in vivo, the actual extent to which the DNA is available to act as a competitor for specific binding (i.e. the effective concentration of nonspecific DNA) is not known. Because many regulatory interactions occur simultaneously in a cell, it is reasonable to expect that they will have evolved to function at equilibrium with a

shared concentration of competing nonspecific DNA. This premise was the basis for this study. In vitro binding data were compiled for six regulatory proteins that function in E. coli, and used to calculate theoretical equilibrium binding distributions. The calculated distributions were used to evaluate the regulatory states of promoters according to models based on the equilibrium occupancies of regulatory sites. For four proteins whose DNA-**binding affinities** are modulated by ligand binding (CAP, lac repressor, trp repressor and araC), regulation was assessed as the extent to which the presence of the modulator could affect the occupancy by protein of the specific sites (e.g. the difference in equilibrium occupancy by CAP of CAP binding sites between conditions of high and low concentrations of CAP's affinity modulator, cAMP). For two proteins whose site affinities are not modulated by ligand binding (λ repressor and λ-cro), regulation was assessed by specific site occupancy at equilibrium. These regulation profiles were compared to determine whether a single concentration of nonspecific competing DNA is compatible with effective regulation as defined for all of the systems. For five of the six modeled systems (CAP, trp repressor, araC, λ repressor and λ-cro), a free nonspecific DNA concentration on the order of 10-4 **M** base pairs is compatible with regulation based on equilibria of the protein-DNA interactions. The lac repressor-operator system is an exception to these results: as has been shown previously, the regulation of operator binding by low molecular weight inducers increases with increasing concentrations of nonspecific DNA (von Hippel et al., 1974 Proc. natn. Acad. Sci. U.S.A. 71, 4808-4812). This discrepancy may indicate that the regulatory state of the lac operator depends on the lifetime of the repressor-operator complex, not simply its equilibrium fractional occupancy. With this exception, the results based on equilibria for the other five systems are consistent with regulation of transcription by protein binding in the presence of an effective DNA concentration [.apprx. **10-4M** base pairs (b.p.)] that is less than 1% of the absolute concentration of total genomic DNA.

L11 ANSWER 5 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
1993:142103 Document No. 118:142103 The biological relevance of the binding of calcium ions by inositol phosphates. Luttrell, Brian M. (Dep. Endocrinol., R. North Shore Hosp., St. Leonards, 2065, Australia). Journal of Biological Chemistry, 268(3), 1521-4 (English) 1993. CODEN: JBCHA3. ISSN: 0021-9258.

AB The binding of Ca2+ (chelation) by myo-inositol polyphosphates at pH 7.0 was studied using a Ca2+-sensitive electrode. Glucose 6-phosphate (used as a model for a monophosphate) bound Ca2+ with an affinity of 152 L/mol and a **molar** ratio of 0.94. Inositol 3,4-bisphosphate, inositol 1,4,5-trisphosphate, inositol 1,3,4,5-tetrakisphosphate, and inositol hexakisphosphate showed affinities of 9.0 + 103, 6.3 + 103, 6.2 + 104, and 1.92 + 105 L/mol, resp., and **molar** ratios of 0.92, 0.95, 0.75, and 2.5. In general, the affinity increased with the number of phosphate substituents on the inositol ring, although the stereochem. is also expected to be important. This suggests that for the physiol. relevant inositol phosphates (tris, tetrakis-, pentakis-, and hexakis-) half-maximal Ca2+ binding will occur in the Ca2+ concentration range of approx. 5 + 10-6 to 2 + **10-4M.** This range lies between the basal intracellular and the free extracellular Ca2+ levels (10-7 and 10-3M), resp., and may therefore be of physiol. importance. Chelation provides a possible simple explanation for the inhibition by Ca2+ of inositol 1,4,5-trisphosphate binding to its receptor in rat cerebellum and other tissues. It may also have a role in limiting inositol phosphate-mediated increases in intracellular Ca2+.

L11 ANSWER 6 OF 9 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
92246936 EMBASE Document No.: 1992246936. Neurokinin A-stimulated

phosphoinositide breakdown in rabbit iris sphincter muscle. Taniguchi T.; Ninomiya H.; Fukunaga R.; Ebii K.; Yamamoto M.; Fujiwara M.. Department of Neurobiology, Kyoto Pharmaceutical University,Kyoto 607, Japan. Japanese Journal of Pharmacology Vol. 59, No. 2, pp. 213-220 1992.
ISSN: 0021-5198. CODEN: JJPAAZ
Pub. Country: Japan. Language: English. Summary Language: English.
ED Entered STN: 920912
AB Specific [3H]-substance P binding was saturable and of high affinity (K(D)=2.5nM) with a B(max) of 725fmol/mg protein in the isolated rabbit iris sphincter muscle. The competition for [3H]substance P binding was in the order of eledoisin>substance P>kassinin>neurokinin B>neurokinin A>physalaemin. In the same preparation, neurokinin A, as well as substance P induced a concentration-related accumulation of [3H]-inositol phosphates (IPs), and the maximum increase was about 200% of the control at **10-4M**. [D-Arg1, D-Trp7,9, Leu11]-substance P (SP) and [D-Pro2, D-Trp7,9]-SP (10-3 **M**) inhibited substance P or neurokinin A (**10-4M**)-induced phosphatidylinositol 4,5-bisphosphate (PIP2) hydrolysis significantly. [D-Arg1, D-Pro2, D-Trp7,9, Leu11]-SP (10-3M) also inhibited neurokinin A (**10-4M**)-induced PIP2 hydrolysis significantly. Neurokinin A and substance P produced concentration-related contractions in normal Ca2+-containing medium. The contractile response was weaker in Ca2+-free medium, and there was no response in 0.2mM EGTA medium. In Ca2+-free medium, the basal level of [3H]-IPs accumulation was smaller than that in normal medium, and neurokinin A and substance P significantly increased PIP2 hydrolysis. In the 0.2 mM EGTA containing medium, neurokinin A and substance P did not stimulate the PIP2 hydrolysis. These results suggest that in the rabbit iris sphincter muscle, there are tachykinin receptors linking to PIP2 hydorolysis and Ca2+ mobilization and that these mechanisms underlie the mechanism for the neurokinin A-induced contractile response, as well as the substance P-induced one.

L11 ANSWER 7 OF 9 CAPLUS COPYRIGHT 2005 ACS on STN
1989:490673 Document No. 111:90673 Effect of cyproterone acetate on aromatase activity in cultured human genital skin fibroblasts: intracellular control of aromatase activity. Fujimoto, Masatoshi (Sch. Med., Yamanashi Med. Coll., Yamanashi, 409-38, Japan). Nippon Naibunpi Gakkai Zasshi, 65(6), 585-602 (Japanese) 1989. CODEN: NNGZAZ. ISSN: 0029-0661.
AB Effects of cyproterone acetate (CA, 10-8-10-5M) on baseline aromatase activity were examined during a 12 h preincubation in the presence of fetal bovine serum (FBS). Basal aromatase activity was not affected by CA, but medroxyprogesterone acetate, a similar synthetic progestogen, induced a 2-fold stimulation of aromatase activity at a concentration of 10-5M. In human genital skin fibroblasts preincubated with dexamethasone (DEX) in the presence of FBS, aromatase activity was stimulated markedly. When the cells were preincubated in the medium containing FBS with DEX (2.5 + 10-7M) in the presence of CA (10-7-**10-4M**), DEX-stimulated levels of aromatase activity were inhibited by CA in a dose-dependent fashion. A competitive binding assay using [3H]DEX, showed that CA competed with DEX for the glucocorticoid receptors and the relative **binding affinity** of CA was approx. 50 times less than DEX. Apparently, the inhibitory effect of CA is due to competition with DEX for receptor binding. Aromatase activity was also stimulated by dibutyryl cAMP [(Bu)2cAMP] (1 mM) in the absence of FBS. The stimulatory effect of (Bu)2cAMP was maximal after 12-24 h of preincubation, and this level was maintained for 60 h. Similar to the DEX stimulation, stimulation of aromatase activity by (Bu)2cAMP required both RNA and protein synthesis, since the stimulatory effect of (Bu)2cAMP was abolished by copreincubation with cycloheximide or actinomycin D. When CA was present during either the 12 h preincubation or assay incubation, no difference was found in the (Bu)2cAMP-stimulated levels of aromatase activity. The non-aromatizable androgen dihydrotestosterone (DHT, 10-8-10-6M) inhibited the stimulation of aromatase activity by (Bu)2cAMP

in a dose-dependent fashion. The inhibitory effect of DHT on (Bu)2cAMP stimulation of aromatase activity was prevented by copreincubation with CA at 10-5 **M**. Addnl. CA probably acts to increase aromatase activity in skin fibroblasts by preventing the inhibitory effect of androgen as antiandrogen under physiol. conditions, although CA inhibits the glucocorticoid stimulation of aromatase activity.

L11 ANSWER 8 OF 9 EMBASE
on STN
82010664 EMBASE Document No.: 1982010664. Properties of an altered dihydrofolate reductase encoded by amplified genes in cultured mouse fibroblasts. Haber D.A.; Beverley S.M.; Kiely M.L.; Schimke R.T.. Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305, United States. Journal of Biological Chemistry Vol. 256, No. 18, pp. 9501-9510 1981.
CODEN: JBCHA3
Pub. Country: United States. Language: English.
ED Entered STN: 911209
AB We have studied a line of 3T6 mouse embryo fibroblasts grown in progressively increasing concentrations of methotrexate. Resistance of cells to low concentrations of the inhibitor (<5 X 10-5M) is attributed to selective multiplication of the genes coding for dihydrofolate reductase and the resulting elevation of enzyme content. Cells isolated at a higher methotrexate concentration (4 X **10-4M**) contain high levels of a dihydrofolate reductase with a reduced affinity for methotrexate. The altered dihydrofolate reductase exhibits a 270-fold reduction in **binding affinity** for methotrexate as measured by equilibrium dialysis (K(d)=5.4 X 10-8M versus 2 X 10-10M for the wild type enzyme). While binding to NADPH is unchanged, the K(**m**) for dihydrofolate is increased 3-fold over wild type enzyme and the turnover number for the reduction of dihydrofolate to tetrahydrofolate is decreased 20-fold. The altered dihydrofolate reductase shows a broader and more predominant acidic peak in its pH profile for this reaction. The molecular weights of the altered and wild type enzymes are identical as determined by sodium dodecyl sulfate-gel electrophoresis, but 2-dimensional electrophoresis reveals a significant basic shift in the migration of the altered enzyme. Studies with various folic acid analogs suggest that modifictions involving the para-aminobenzoyl moiety of the inhibitor molecules are associated with the most dramatic differential binding between the altered and wild type dihydrofolate reductases.

L11 ANSWER 9 OF 9 EMBASE
on STN DUPLICATE 1
78342396 EMBASE Document No.: 1978342396. Neurohypophyseal hormone-responsive renal adenylate cyclase. II. Relationship between hormonal occupancy of neurohypophyseal hormone receptor sites and adenylate cyclase activation. Hechter O.; Terada S.; Nakahara T.; et al.. Dept. Physiol., Northwest. Univ. Med. Cent., Chicago, Ill. 60611, United States. Journal of Biological Chemistry Vol. 253, No. 9, pp. 3219-3229 1978.
CODEN: JBCHA3
Pub. Country: United States. Language: English.
AB Specific binding sites for 3H-labeled [Lys8]vasopressin (LVP) or 3H-labeled [Arg8]vasopressin (AVP) as ligand have been detected in a bovine renal medullary membrane preparation, incubated under standard 'optimal' conditions previously shown to maximize the sensitivity and responsiveness of adenylate cyclase to neurohypophyseal hormones (NHH). Specific binding involves in limited number of high affinity binding sites, which appear to be homogeneous as evidenced by linearity of Scatchard plots and by the similarity of the K(d) values for [3H]LVP binding as evaluated independently from equilibrium saturation and kinetic data. The specific membrane sites bind [3H]NHH reversibly in noncooperative fashion (Hill coefficient near 1.0). These sites are differentiated from a large number of low affinity 'nonspecific' sites which bind [3H]AVP and [3H]LVP in the presence of very high concentrations of unlabeled hormone (10-4 **M**). The total number of specific

binding sites (B(max)) varied from 1.3 to 4.0 pmol of [3H]LVP (or[3H]AVP) per mg of protein; the [3H]LVP concentration required for half-maximal binding (K(b)) varied from 5 to 25 nM. The half-maximal [3H]LVP concentration for adenylate cyclase activation (K(a)) was uniformly lower, the ratio of K(b)/K(a) varying from 3.7 to 6.2. The ratio of B(max)/V(max) (velocity of maximally activated NHH-specific adenylate cyclase) in these membranes likewise varied by a factor of 4. The specificity of hormone specific binding sites was evaluated by competitive binding with unlabeled peptides and [3H]LVP. Unlabeled AVP, [Arg8]vasotocin (AVT), LVP, oxytocin (OT), deaminopressinamide (DeP), and deaminotocinamide (DeT) completely inhibited the specific binding of [3H]LVP; the apparent K(b) value for each peptide was calculated from the concentration required to produce 50% inhibition (H(50)) relative to unlabeled LVP. The apparent **binding affinity** (1/K(b)) for specific sites had the same order (AVP>AVT>LVP>>OT>>DeP>DeT) as that observed for adenylate cyclase activation. There is a striking linear relationship between log K(b) and log K(a), suggesting that each of these peptide molecules occupies the same 'pocket' in a single class of receptor molecules. Insulin, angiotensin, glucagon, and the ACTH5-10 fragment (which do not stimulate NHH-specific adenylate cyclase) significantly inhibited [3H]LVP binding only at very high concentrations (exceeding **10-4M**).

```
=> s (brieseqitz r?/au or crabtree g?/au or wandless t?/au)
L12       2443 (BRIESEQITZ R?/AU OR CRABTREE G?/AU OR WANDLESS T?/AU)

=> s l12 and targeting
L13         49 L12 AND TARGETING

=> dup remove l3
PROCESSING COMPLETED FOR L3
L14         74 DUP REMOVE L3 (43 DUPLICATES REMOVED)

=> dup remove l13
PROCESSING COMPLETED FOR L13
L15         36 DUP REMOVE L13 (13 DUPLICATES REMOVED)

=> d l15 1-36 cbib abs

L15  ANSWER 1 OF 36  CAPLUS  COPYRIGHT 2005 ACS on STN
2005:120680  Document No. 142:191301  Neurodegenerative protein aggregation
     inhibition methods using compounds comprising a targeting
     element and a recruiting element.  Graef, Isabella A.; Crabtree,
     Gerald R.; Gestwicki, Jason E. (The Board of Trustees of the Leland
     Stanford Junior University, USA).  PCT Int. Appl. WO 2005011610 A2
     20050210, 36 pp.  DESIGNATED STATES: W:  AE, AG, AL, AM, AT, AU, AZ, BA,
     BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC,
     EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG,
     KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ,
     NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY,
     TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW; RW: AT,
     BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE,
     IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR.  (English).  CODEN:
     PIXXD2.  APPLICATION: WO 2004-US24929 20040729.  PRIORITY: US
     2003-PV491482 20030730.
AB   Methods and compns. are provided for reducing aggregation of
     neurodegenerative proteins associated with neurotoxicity or other proteins.
     The compds. comprise a first domain or targeting element for
     binding to the target proteins linked to a second domain or recruiting
     element that binds to an aggregation inhibiting protein, e.g. a prolyl
     isomerase.  The compds. have the formula R1-L-R2, wherein R1 is selected
     from the group consisting of 1-Br or I-2,5-bis(3-hydroxycarbonyl-4-
     hydroxy)stilbene, thioflavin, thioflavin T, chrysamine G, X-34, Congo red,
```

IMPY, imipramine, carbamazepine, phenazine, phenothiazine, promazine, chloropromazine, haloperidol, clozapine, 2-chlorophenothiazine, promethazine, chloroprothixen, acepromazine, deoxydoxorubicin, rifamycin, acridone and acridone derivs., such as flavonoids and alkaloids, benzofurans, 9-substituted acridines, pamaquine, chloroquine and amacrine, and methylene blue; L is a bond or linking group of not more than about 36 atoms other than hydrogen; and R2 is selected from the group consisting of SLF, FK506 and rapamycin. By associating the aggregating forming proteins or neuronal cells under conditions where aggregating proteins are produced with the compound and the aggregation inhibiting protein, aggregation is reduced. The subject agents can be used in assays, investigating the etiol. of the neuronal diseases and for prophylaxis and therapy.

L15 ANSWER 2 OF 36 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2004265453 EMBASE Genetic loss of calcineurin blocks mechanical overload-induced skeletal muscle fiber type switching but not hypertrophy. Parsons S.A.; Milla D.P.; Wilkins B.J.; Bueno O.F.; Tsika G.L.; Neilson J.R.; Liberatore C.M.; Yutzey K.E.; **Crabtree G.R.**; Tsika R.W.; Molkentin J.D.. J.D. Molkentin, Div. of Molec. Cardiovasc. Biology, Cincinnati Children's Hosp. Med. C., 3333 Burnet Ave., Cincinnati, OH 45229-3039, United States. jeff.molkentin@cchmc.org. Journal of Biological Chemistry Vol. 279, No. 25, pp. 26192-26200 18 Jun 2004.
Refs: 69.
ISSN: 0021-9258. CODEN: JBCHA3
Pub. Country: United States. Language: English. Summary Language: English.

ED Entered STN: 20040722

AB The serine/threonine phosphatase calcineurin is an important regulator of calcium-activated intracellular responses in eukaryotic cells. In higher eukaryotes, calcium/calmodulin-mediated activation of calcineurin facilitates direct dephosphorylation and nuclear translocation of the transcription factor nuclear factor of activated T-cells (NFAT). Recently, controversy has surrounded the role of calcineurin in mediating skeletal muscle cell hypertrophy. Here we examined the ability of calcineurin-deficient mice to undergo skeletal muscle hypertrophic growth following mechanical overload (MOV) stimulation or insulin-like growth factor-1 (IGF-1) stimulation. Two distinct models of calcineurin deficiency were employed: calcineurin Aβ gene-targeted mice, which show a .apprx.50% reduction in total calcineurin, and calcineurin B1-LoxP-targeted mice crossed with a myosin light chain 1f cre knock-in allele, which show a greater than 80% loss of total calcineurin only in skeletal muscle. Calcineurin Aβ-/- and calcineurin B1-LoxP(fl/fl)-MLC-cre mice show essentially no defects in muscle growth in response to IGF-1 treatment or MOV stimulation, although calcineurin Aβ-/- mice show a basal defect in total fiber number in the plantaris and a mild secondary reduction in growth, consistent with a developmental defect in myogenesis. Both groups of gene-targeted mice show normal increases in Akt activation following MOV or IGF-1 stimulation. However, overload-mediated fiber-type switching was dramatically impaired in calcineurin B1-LoxP(fl/fl)-MLC-cre mice. NFAT-luciferase reporter transgenic mice failed to show a correlation between IGF-1- or MOV-induced hypertrophy and calcineurin-NFAT-dependent signaling in vivo. We conclude that calcineurin expression is important during myogenesis and fiber-type switching, but not for muscle growth in response to hypertrophic stimuli.

L15 ANSWER 3 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN

2004:226546 Bifunctional small molecules as inhibitors of amyloidogenic protein-protein interactions. Gestwicki, Jason E.; Graef, Isabella; **Crabtree, Gerald R.** (Department of Pathology, Stanford University School of Medicine, Stanford, CA, 94305, USA). Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA, United States, March 28-April 1, 2004, MEDI-188. American Chemical Society: Washington, D. C. (English) 2004. CODEN: 69FGKM.

AB The deposition of amyloidogenic peptide fibrils is the hallmark of

numerous neurodegenerative diseases, such as Alzheimer's and Parkinson's. In Alzheimer's disease, fibrils are assembled as a result of progressive protein-protein interactions between copies of the amyloid beta (Ab) peptide. Small mols. that effectively bind Ab have been generated, but these compds. typically have disappointing inhibition consts. It is likely that small mols. lack sufficient steric bulk to prevent interactions between relatively large peptide surfaces. Mindful of these considerations, we report a strategy that takes advantage of bifunctional mols. to recruit endogenous cellular proteins to aggregating Ab. These compds. are comprised of an Ab **targeting** moiety and a recruitment domain that interacts with the ubiquitous cellular protein, FKBP. In the presence of FKBP, our inhibitors are at least 5-fold more potent than equivalent small mols. Thus, these compds. are among the most effective inhibitors of Ab aggregation reported.

L15 ANSWER 4 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
2004:14778 Document No. 140:194081 Conditional protein alleles using knockin mice and a chemical inducer of dimerization. Stankunas, Kryn; Bayle, J. Henri; Gestwicki, Jason E.; Lin, Yun-ming; **Wandless, Thomas J.**; **Crabtree, Gerald R.** (Departments of Developmental Biology and Pathology Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA, 94305, USA). Molecular Cell, 12(6), 1615-1624 (English) 2003. CODEN: MOCEFL. ISSN: 1097-2765. Publisher: Cell Press.
AB The authors have developed a general method of making conditional alleles that allows the rapid and reversible regulation of specific proteins. A mouse line was produced in which proteins encoded by the endogenous glycogen synthase kinase-3 β (GSK-3β) gene are fused to an 89 amino acid tag, FRB*. FRB* causes the destabilization of GSK-3β, producing a severe loss-of-function allele. In the presence of C20-MaRap, a highly specific, nontoxic, cell-permeable small mol., GSK-3βFRB* binds to the ubiquitously expressed FKBP12 protein. This interaction stabilizes GSK-3βFRB* and restores both protein levels and activity. C20-MaRap-mediated stabilization is rapidly reversed by the addition of an FKBP12 binding competitor mol. This technol. may be applied to a wide range of FRB*-tagged mouse genes while retaining their native transcriptional control. Inducible stabilization could be valuable for many developmental and physiol. studies and for drug target validation.

L15 ANSWER 5 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 1
2002:218987 Document No.: PREV200200218987. NF-AT polypeptides and polynucleotides and screening methods for immunosuppressive agents. **Crabtree, Gerald R.** [Inventor, Reprint author]; Northrop, Jeffrey P. [Inventor]; Ho, Steffan N. [Inventor]; Flanagan, William M. [Inventor]. Woodside, CA, USA. ASSIGNEE: The Board of Trustees of the Leland Stanford Junior University. Patent Info.: US 6352830 March 05, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 5, 2002) Vol. 1256, No. 1. http://www.uspto.gov/web/menu/patdata.html. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging

of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals. Also included are methods and agents for activation of NF-AT dependent transcription, including agents which interfere with the production, modification of nuclear or cytoplasmic subunits, or the nuclear import of the cytoplasmic subunits. In particular, screening tests for novel immunosuppressants are provided based upon the ability of NF-AT to activate transcription.

L15 ANSWER 6 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2002:338544 Document No.: PREV200200338544. NF-AT polypeptides and polynucleotides. **Crabtree, Gerald R.** [Inventor]; Northrop, Jeffrey P. [Inventor]; Ho, Steffan N. [Inventor, Reprint author]. Menlo Park, CA, USA. ASSIGNEE: Board of Trustees of the Leland Stanford Junior University, Stanford, CA, USA. Patent Info.: US 6388052 May 14, 2002. Official Gazette of the United States Patent and Trademark Office Patents, (May 14, 2002) Vol. 1258, No. 2. http://www.uspto.gov/web/menu/patdata.htm l. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals.

L15 ANSWER 7 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 2
2001:383566 Document No.: PREV200100383566. NF-AT polypeptides and polynucleotides. **Crabtree, Gerald R.** [Inventor]; Northrop, Jeffrey P. [Inventor]; Ho, Steffan N. [Inventor]. ASSIGNEE: Sara Lee Corporation. Patent Info.: US 6197925 March 06, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Mar. 6, 2001) Vol. 1244, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies

reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals.

L15 ANSWER 8 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 3
2001:334722 Document No.: PREV200100334722. NF-AT polypeptides and polynucleotides. **Crabtree, Gerald R.** [Inventor]; Northrop, Jeffrey P. [Inventor, Reprint author]; Ho, Steffan N. [Inventor]. Cupertino, CA, USA. ASSIGNEE: The Board of Trustees of the Leland Stanford Junior University. Patent Info.: US 6171781 January 09, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Jan. 9, 2001) Vol. 1242, No. 2. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals.

L15 ANSWER 9 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
2002:39523 Document No.: PREV200200039523. NF-AT polypeptides and polynucleotides. **Crabtree, Gerald R.** [Inventor]; Northrop, Jeffrey P. [Inventor]; Ho, Steffan N. [Inventor]. ASSIGNEE: Board of Trustees of the Leland Stanford Junior University. Patent Info.: US 6312899 November 06, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 6, 2001) Vol. 1252, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.
AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals.

L15 ANSWER 10 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
2001:380753 Document No. 134:361402 Bifunctional inhibitor molecules, their use in the disruption of protein-protein interactions and therapeutic applications. **Crabtree, Gerald R.**; Stankunas, Kryn; Briesewitz, Roger; **Wandless, Thomas** (The Board of Trustees of the Leland

Stanford Junior University, USA). PCT Int. Appl. WO 2001036612 A1 20010525, 30 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31695 20001117. PRIORITY: US 1999-PV166675 19991119.

AB Bifunctional inhibitor mols. and methods for their use in the inhibition of protein-protein interactions are provided. The subject bifunctional inhibitor mols. are conjugates of a target protein ligand and a blocking protein ligand, where these two moieties are optionally joined by a linking group. In the subject methods, an effective amount of the bifunctional inhibitor mol. is administered to a host in which the inhibition of a protein-protein interaction is desired. The bifunctional inhibitor mol. simultaneously binds to its corresponding target and blocking proteins to produce a tripartite complex that inhibits the target protein-protein interaction. The subject methods and compns. find use in a variety of applications, including therapeutic applications.

L15 ANSWER 11 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN

2001:380414 Document No. 134:371812 Targeted bifunctional molecules and therapies based thereon. Briesewitz, Roger; **Crabtree, Gerald R.** ; **Wandless, Thomas** (Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 2001035978 A1 20010525, 31 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US31702 20001117. PRIORITY: US 1999-PV166580 19991119.

AB Targeted bifunctional mols. and methods for their use are provided. The subject targeted bifunctional mols. are conjugates of a drug moiety and a **targeting** moiety, where these two moieties are optionally joined by a linking group. The bifunctional mols. are further characterized in that they exhibit a modulated biodistribution upon administration to a host as compared to a free drug control. The subject targeted bifunctional mols. find use in a variety of therapeutic applications. For example, a bifunctional mol. consisting of a drug moiety covalently joined to sulfisoxazole which is extensively bound by albumin, via an inert linking group is formed. When this bifunctional mol. enters the human circulation, it is bound by albumin which keeps the drug of interest in the extracellular environment.

L15 ANSWER 12 OF 36 MEDLINE on STN

2001385235. PubMed ID: 11439183. Signals transduced by Ca(2+)/calcineurin and NFATc3/c4 pattern the developing vasculature. Graef I A; Chen F; Chen L; Kuo A; **Crabtree G R.** (Department of Developmental Biology, Howard Hughes Medical Institute, Stanford University, Stanford, CA 94305, USA.) Cell, (2001 Jun 29) 105 (7) 863-75. Journal code: 0413066. ISSN: 0092-8674. Pub. country: United States. Language: English.

AB Vascular development requires an orderly exchange of signals between growing vessels and their supporting tissues, but little is known of the intracellular signaling pathways underlying this communication. We find that mice with disruptions of both NFATc4 and the related NFATc3 genes die around E11 with generalized defects in vessel assembly as well as excessive and disorganized growth of vessels into the neural tube and somites. Since calcineurin is thought to control nuclear localization of

NFATc proteins, we introduced a mutation into the calcineurin B gene that prevents phosphatase activation by Ca(2+) signals. These CnB mutant mice exhibit vascular developmental abnormalities similar to the NFATc3/c4 null mice. We show that calcineurin function is transiently required between E7.5 and E8.5. Hence, early calcineurin/NFAT signaling initiates the later cross-talk between vessels and surrounding tissues that pattern the vasculature.

L15 ANSWER 13 OF 36 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2001154079 EMBASE Calcineurin inhibitors and the generalization of the presenting protein strategy. Vogel K.W.; Briesewitz R.; **Wandless T.J.**; **Crabtree G.R.**. K.W. Vogel, MAXYGEN, 515 Galveston Drive, Redwood City, CA 94063, United States. Advances in Protein Chemistry Vol. 56, pp. 253-291 2001.
Refs: 120.
ISSN: 0065-3233. CODEN: APCHA2
Pub. Country: United States. Language: English.

ED Entered STN: 20010517
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L15 ANSWER 14 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:265557 Document No.: PREV200100265557. NF-AT polypeptides and polynucleotides. **Crabtree, Gerald R.** [Inventor]; Northrop, Jeffrey P. [Inventor]; Ho, Steffan N. [Inventor]. ASSIGNEE: Board of Trustees of the Leland Stanford Junior University. Patent Info.: US 6150099 November 21, 2000. Official Gazette of the United States Patent and Trademark Office Patents, (Nov. 21, 2000) Vol. 1240, No. 3. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals.

L15 ANSWER 15 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2001:177889 Document No.: PREV200100177889. NF-AT polynucleotides. **Crabtree, Gerald R.** [Inventor]; Northrop, Jeffrey P. [Inventor, Reprint author]; Ho, Steffan N. [Inventor]. Campbell, CA, USA. ASSIGNEE: Board of Trustees of the Leland Stanford Junior University, Stanford, CA, USA. Patent Info.: US 6096515 August 01, 2000. Official Gazette of the United States Patent and Trademark Office Patents, (Aug. 1, 2000) Vol. 1237, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode

such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as research or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals.

L15 ANSWER 16 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

2000:439290 Document No.: PREV200000439290. Regulated transcription of targeted genes and other biological events. **Crabtree, Gerald R.** [Inventor]; Schreiber, Stuart L. [Inventor]; Spencer, David M. [Inventor]; **Wandless, Thomas J.** [Inventor]; Belshaw, Peter [Inventor]; Ho, Steffan N. [Inventor]. ASSIGNEE: Board of Trustees of Leland Stanford Jr. University, Stanford, CA, USA; President and Fellows of Harvard College. Patent Info.: US 6046047 April 04, 2000. Official Gazette of the United States Patent and Trademark Office Patents, (Apr. 4, 2000) Vol. 1233, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Dimerization and oligomerization of proteins are general biological control mechanisms that contribute to the activation of cell membrane receptors, transcription factors, vesicle fusion proteins, and other classes of intra- and extracellular proteins. We have developed a general procedure for the regulated (inducible) dimerization or oligomerization of intracellular proteins. In principle, any two target proteins can be induced to associate by treating the cells or organisms that harbor them with cell permeable, synthetic ligands. To illustrate the practice of tis invention, we have induced: (1) the intracellular aggregation of the cytoplasmic tail of the zeta chain of the T cell receptor (TCR)-CD3 complex thereby leading to signaling and transcription of a reporter gene, (2) the homodimerization of the cytoplasmic tail of the Fas receptor thereby leading to cell-specific apoptosis (programmed cell death) and (3) the heterodimerization of a DNA-binding domain (Gal4) and a transcription-activation domain (VP16) thereby leading to direct transcription of a reporter gene. Regulated intracellular protein association with our cell permeable, synthetic ligands offers new capabilities in biological research and medicine, in particular, in gene therapy. Using gene transfer techniques to introduce our artificial receptors, one can turn on or off the signaling pathways that lead to the overexpression of therapeutic proteins by administering orally active "dimerizers" or "de-dimerizers", respectively. Since cells from different recipients can be configured to have the pathway overexpress different therapeutic proteins for use in a variety of disorders, the dimerizers have the potential to serve as "universal drugs". They can also be viewed as cell permeable, organic replacements for therapeutic antisense agents or for proteins that would otherwise require intravenous injection or intracellular expression (e.g., the LDL receptor or the CFTR protein).

L15 ANSWER 17 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN

2000:368133 Document No. 133:12751 NF-AT mediates cardiac hypertrophy, methods and reagents related thereto. **Crabtree, Gerald R.**; Northrop, Jeffrey P.; Ho, Steffan N. (The Board of Trustees of the Leland Stanford Jr. University, USA). PCT Int. Appl. WO 2000030671 A2 20000602, 138 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1999-US27862 19991123. PRIORITY: US 1998-198977 19981124.

AB The invention is a method for treating cardiac hypertrophy, or preventing other growth of cardiac and vascular tissue, through the use of NF-AT (nuclear factor of activated T cells) antagonists.

L15 ANSWER 18 OF 36 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
2000234705 EMBASE A confederacy of bunches: Fundamentals and applications of a self- associating protein. **Wandless T.J.**. T.J. Wandless, Department of Chemistry, Stanford University, Stanford, CA 94305, United States. wandless@chem.stanford.edu. Proceedings of the National Academy of Sciences of the United States of America Vol. 97, No. 13, pp. 6921-6923 20 Jun 2000.
Refs: 12.
ISSN: 0027-8424. CODEN: PNASA6
Pub. Country: United States. Language: English.
ED Entered STN: 20000720
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L15 ANSWER 19 OF 36 MEDLINE on STN DUPLICATE 4
2001154203. PubMed ID: 11163203. A Brg1 null mutation in the mouse reveals functional differences among mammalian SWI/SNF complexes. Bultman S; Gebuhr T; Yee D; La Mantia C; Nicholson J; Gilliam A; Randazzo F; Metzger D; Chambon P; **Crabtree G**; Magnuson T. (Department of Genetics, Case Western Reserve University, Cleveland, OH 44106, USA.) Molecular cell, (2000 Dec) 6 (6) 1287-95. Journal code: 9802571. ISSN: 1097-2765. Pub. country: United States. Language: English.
AB Mammalian SWI/SNF complexes utilize either brahma (Brm) or brahma-related gene 1 (Brg1) catalytic subunits to remodel nucleosomes in an ATP-dependent manner. Brm was previously shown to be dispensable, suggesting that Brm and Brg1 are functionally redundant. To test this hypothesis, we have generated a Brg1 null mutation by gene **targeting**, and, surprisingly, homozygotes die during the periimplantation stage. Furthermore, blastocyst outgrowth studies indicate that neither the inner cell mass nor trophectoderm survives. However, experiments with other cell types demonstrate that Brg1 is not a general cell survival factor. In addition, Brg1 heterozygotes are predisposed to exencephaly and tumors. These results provide evidence that biochemically similar chromatin-remodeling complexes have dramatically different functions during mammalian development.

L15 ANSWER 20 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
1999:180895 Document No. 130:346784 Affinity modulation of small-molecule ligands by borrowing endogenous protein surfaces. Briesewitz, Roger; Ray, Gregory T.; **Wandless, Thomas J.**; **Crabtree, Gerald R.** (Howard Hughes Medical Institute, Stanford University, Stanford, CA, 94305, USA). Proceedings of the National Academy of Sciences of the United States of America, 96(5), 1953-1958 (English) 1999. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB A general strategy is described for improving the binding properties of small-mol. ligands to protein targets. A bifunctional mol. is created by chemical linking a ligand of interest to another small mol. that binds tightly to a second protein. When the ligand of interest is presented to the target protein by the second protein, addnl. protein-protein interactions outside of the ligand-binding sites serve either to increase or decrease the affinity of the binding event. We have applied this approach to an intractable target, the SH2 domain, and demonstrate a 3-fold enhancement over the natural peptide. This approach provides a way to modulate the potency and specificity of biol. active compds.

L15 ANSWER 21 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
1999:204203 Document No. 130:350038 Generic signals and specific outcomes: signaling through Ca2+, calcineurin, and NF-AT. **Crabtree, Gerald R.** (Department of Pathology Department of Development Biology, Stanford University Medical School, Stanford, CA, 94062, USA). Cell

(Cambridge, Massachusetts), 96(5), 611-614 (English) 1999. CODEN: CELLB5. ISSN: 0092-8674. Publisher: Cell Press.

AB A review with 25 refs., addressing the puzzle of signal specificity by way of the example of Ca2+, calcineurin, and NF-AT signaling in the heart. Discussed are tissue-specific expression of NF-ATc family members; cellular context as a means of imparting signal specificity from generic signals; and hypotheses on how a ubiquitous signaling intermediate can become a drug-specific therapeutic target.

L15 ANSWER 22 OF 36 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2000189956 EMBASE Signaling through calcium, calcineurin, and NF-AT in lymphocyte activation and development. Stankunas K.; Graef I.A.; Neilson J.R.; Park S.-H.; **Crabtree G.R.**. K. Stankunas, Department of Developmental Biology, Howard Hughes Medical Institute, Stanford University Medical School, Stanford, CA 94305, United States. Cold Spring Harbor Symposia on Quantitative Biology Vol. 64, pp. 505-516 1999. Refs: 85.
ISSN: 0091-7451. CODEN: CSHSAZ
Pub. Country: United States. Language: English.

ED Entered STN: 20000622
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L15 ANSWER 23 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN

1999:267043 Document No. 131:83012 Molecular composition and biophysical characteristics of nicotinic receptors. Ramirez-Latorre, J.; **Crabtree, G.**; Turner, Jennifer; Role, Lorna (Department of Biology, Temple University, Philadelphia, PA, USA). Neuronal Nicotinic Receptors, 43-64. Editor(s): Arneric, Stephen P.; Brioni, Jorge D. Wiley-Liss: New York, N. Y. (English) 1999. CODEN: 670UAH.

AB A review, with 132 refs., on the subunit composition and consequent biophys. properties of nicotinic receptors. The authors specifically considered the physiol. of recombinant nicotinic receptors of known composition and the mol. determinants of native nicotinic receptors, native nicotinic receptor subtypes, and **targeting** of nicotinic receptors to subcellular domains.

L15 ANSWER 24 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN

1998:734993 Document No. 130:1173 Regulated apoptosis by chimeric proteins binding to FK506-type and cyclosporin-type ligands. **Crabtree, Gerald R.**; Schreiber, Stuart L.; Spencer, David M.; **Wandless, Thomas J.**; Belshaw, Peter (President & Fellows of Harvard College, USA; Board of Trustees of Leland Stanford Jr. University). U.S. US 5834266 A 19981110, 104 pp., Cont.-in-part of U.S. Ser. No. 179,143, abandoned. (English). CODEN: USXXAM. APPLICATION: US 1994-292597 19940818. PRIORITY: US 1993-17931 19930212; US 1993-92977 19930716; US 1993-93499 19930716; US 1994-179143 19940107; US 1994-179748 19940107.

AB A general procedure is described for the regulated (inducible) dimerization or oligomerization of intracellular proteins and methods and materials are presented for using that procedure to regulatably initiate cell-specific apoptosis (programmed cell death) in genetically engineered cells. The procedure involves chimeric (or fused) proteins, DNA constructs encoding them, and ligand mols. capable of oligomerizing the chimeric proteins. The chimeric proteins contain at least one ligand-binding (or receptor) domain fused to an action domain capable of initiating apoptosis within a cell (e.g., Fas or tumor necrosis factor receptor), and may also contain addnl. domains for (1) the regulatable or constitutive expression of desired genes and (2) intracellular **targeting.** The chimeric proteins are capable of binding to an FK506-type ligand, a cyclosporin A-type ligand, tetracycline, or a steroid ligand. One such chimeric protein is myristoylated CD3/FKBP12 (MZF3E) receptor consisting of (1) a c-src fragment sufficient for myristoylation, (2) the cytoplasmic tail of ζ (a component of the B cell receptor), (3) 3 consecutive domains of the FKBP12 immunophilin, and (4) a flu

epitope tag; oligomerization/apoptosis is induced by a dimeric derivative of FK506. Syntheses are reported for the preparation of dimeric and "bumped" (containing steric bulky groups) derivs. of FK506 and cyclosporin A. The overall procedures allows ligand-mediated oligomerization for regulated gene therapy.

L15 ANSWER 25 OF 36 SCISEARCH COPYRIGHT (c) 2005 The Thomson Corporation on STN

1998:907759 The Genuine Article (R) Number: 137GQ. Pip2-dependent **targeting** of the mammalian SWI/SNF or BAF complex to chromatin during lymphocyte activation. Zhao K (Reprint); Wang W; Rando O J; **Crabtree G R.** STANFORD UNIV, HOWARD HUGHES MED INST, STANFORD, CA 94305; STANFORD UNIV, DEPT DEV BIOL, STANFORD, CA 94305. MOLECULAR BIOLOGY OF THE CELL (NOV 1998) Vol. 9, Supp. [S], pp. 1852-1852. Publisher: AMER SOC CELL BIOLOGY. PUBL OFFICE, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 1059-1524. Pub. country: USA. Language: English.

L15 ANSWER 26 OF 36 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN

1999:19261 Document No.: PREV199900019261. PIP2-dependent **targeting** of the mammalian SWI/SNF or BAF complex to chromatin during lymphocyte activation. Zhao, K.; Wang, W.; Rando, O. J.; **Crabtree, G. R..** HHMI Dep. Dev. Biol., Stanford Univ., Stanford, CA 94305, USA. Molecular Biology of the Cell, (Nov., 1998) Vol. 9, No. SUPPL., pp. 319A. print. Meeting Info.: 38th Annual Meeting of the American Society for Cell Biology. San Francisco, California, USA. December 12-16, 1998. American Society for Cell Biology.
CODEN: MBCEEV. ISSN: 1059-1524. Language: English.

L15 ANSWER 27 OF 36 MEDLINE on STN

1998175718. PubMed ID: 9515963. Role of the NF-ATc transcription factor in morphogenesis of cardiac valves and septum. de la Pompa J L; Timmerman L A; Takimoto H; Yoshida H; Elia A J; Samper E; Potter J; Wakeham A; Marengere L; Langille B L; **Crabtree G R**; Mak T W. (The Amgen Institute, Department of Medical Biophysics, University of Toronto, Ontario, Canada.) Nature, (1998 Mar 12) 392 (6672) 182-6. Journal code: 0410462. ISSN: 0028-0836. Pub. country: ENGLAND: United Kingdom. Language: English.

AB In lymphocytes, the expression of early immune response genes is regulated by NF-AT transcription factors which translocate to the nucleus after dephosphorylation by the Ca2+-dependent phosphatase, calcineurin. We report here that mice bearing a disruption in the NF-ATc gene fail to develop normal cardiac valves and septa and die of circulatory failure before day 14.5 of development. NF-ATc is first expressed in the heart at day 7.5, and is restricted to the endocardium, a specialized endothelium that gives rise to the valves and septum. Within the endocardium, specific inductive events appear to activate NF-ATc: it is localized to the nucleus only in endocardial cells that are adjacent to the interface with the cardiac jelly and myocardium, which are thought to give the inductive stimulus to the valve primordia. Treatment of wild-type embryos with FK506, a specific calcineurin inhibitor, prevents nuclear localization of NF-ATc. These data indicate that the Ca2+/calcineurin/NF-ATc signalling pathway is essential for normal cardiac valve and septum morphogenesis; hence, NF-ATc and its regulatory pathways are candidates for genetic defects underlying congenital human heart disease.

L15 ANSWER 28 OF 36 MEDLINE on STN DUPLICATE 5

97431685. PubMed ID: 9285717. Rapid **targeting** of nuclear proteins to the cytoplasm. Klemm J D; Beals C R; **Crabtree G R.** (Howard Hughes Medical Institute, Department of Developmental Biology, Stanford University School of Medicine, Stanford, California 94305, USA.) Current biology : CB, (1997 Sep 1) 7 (9) 638-44. Journal code: 9107782. ISSN: 0960-9822. Pub. country: ENGLAND: United Kingdom. Language: English.

AB BACKGROUND: The transcription factor NF-ATc plays a key role in the

activation of many early immune response genes and is regulated by subcellular localization. NF-ATc translocates from the cytoplasm to the nucleus in response to a rise in intracellular calcium, and immediately returns to the cytoplasm when intracellular calcium levels fall. The rapid nuclear exit of NF-ATc is thought to be one mechanism by which cells distinguish between sustained and transient calcium signals. RESULTS: To study the nuclear export of NF-ATc, we have developed a general, non-invasive assay for the identification and study of nuclear export signals (NESs). The NES is defined by its ability to translocate a protein from the nucleus to the cytoplasm when the two are tethered by a membrane-permeable ligand. This procedure has allowed us to identify a NES within NF-ATc that functions in concert with a glycogen synthase kinase-regulated process to direct the rapid nuclear exit of NF-ATc. CONCLUSIONS: The rapid nuclear export of NF-ATc via its NES and a glycogen synthase kinase-regulated event may be an important mechanism for insulating cells from transient spikes in intracellular calcium which might otherwise lead to inappropriate activation. The assay we have developed allows the rapid identification of NESs and can be used as a general method for the inducible cytoplasmic export of nuclear proteins.

L15 ANSWER 29 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
1996:350220 Document No. 125:27701 Regulatable elimination of gene expression, gene product function and engineered host cells, and its application in gene therapy. Brugge, Joan S.; **Crabtree, Gerald R.** (Ariad Gene Therapeutics, Inc., USA). PCT Int. Appl. WO 9606111 A1 19960229, 141 pp. DESIGNATED STATES: W: AU, CA, GB, JP, KR, US; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1995-US10591 19950818. PRIORITY: US 1994-292595 19940818; US 1994-292596 19940818; US 1994-292597 19940818.
AB Materials and methods are disclosed for regulated obstruction of the expression of a target gene or the biol. effect of its gene product in genetically engineered cells or organisms containing them. Aspects of the invention are exemplified by recombinant modifications of host cells and their use in vitro and in vivo for the regulatable blockade of expression of a target gene, for interference with the function or effect of a target gene product or for the regulatable elimination of a target gene. Synthesis of oligomer of ligands such as FK506 and cyclosporin A, and regulation of programmed cell death with immunophilin-Fas antigen chimeras were demonstrated.

L15 ANSWER 30 OF 36 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
96161573 EMBASE Document No.: 1996161573. Controlling protein association and subcellular localization with a synthetic ligand that induces heterodimerization of proteins. Belshaw P.J.; Ho S.N.; **Crabtree G.R.**; Schreiber S.L.. Dept. of Pathology/Devtl. Biology, Howard Hughes Medical Institute, Stanford University Sch. of Medicine,Stanford, CA 94305, United States. Proceedings of the National Academy of Sciences of the United States of America Vol. 93, No. 10, pp. 4604-4607 1996. ISSN: 0027-8424. CODEN: PNASA6
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 960617
AB Extracellular growth and differentiation factors induce changes in gene expression in the nucleus by initiating a series of protein associations that alter the subcellular localization of intracellular signaling proteins. Initial events involve receptor homo- or heterodimerization and subsequent recruitment of cytosolic signaling proteins to the inner leaflet of the plasma membrane. Intermediate events involve the translocation of proteins into the nucleus. Late events involve the recruitment of transcriptional activators to the vicinity of specific genes in the nucleus, resulting in increased gene transcription. The ability to induce signals at each of these three phases of signaling pathways is illustrated by the use of a heterodimeric chemical inducer of dimerization that causes a proximal relationship between two different

target proteins.

L15 ANSWER 31 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
1995:659629 Document No. 123:49272 NF-AT polypeptides and polynucleotides and method for detection of T cells. **Crabtree, Gerald R.**; Northrop, Jeffrey P.; Ho, Steffan N. (Board of Trustees of the Leland Stanford Junior University, USA). PCT Int. Appl. WO 9508554 A1 19950330, 85 pp. DESIGNATED STATES: W: AU, CA, JP; RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US10724 19940920. PRIORITY: US 1993-124981 19930920; US 1994-260174 19940613.

AB The invention provides novel polypeptides which are associated with the transcription complex NF-AT, polynucleotides encoding such polypeptides, antibodies which are reactive with such polypeptides, polynucleotide hybridization probes and PCR amplification probes for detecting polynucleotides which encode such polypeptides, transgenes which encode such polypeptides, homologous **targeting** constructs that encode such polypeptides and/or homologously integrate in or near endogenous genes encoding such polypeptides, nonhuman transgenic animals which comprise functionally disrupted endogenous genes that normally encode such polypeptides, and transgenic nonhuman animals which comprise transgenes encoding such polypeptides. The invention also provides methods for detecting T cells (including activated T cells) in a cellular sample, methods for treating hyperactive or hypoactive T cell conditions, methods for screening for immunomodulatory agents, methods for diagnostic staging of lymphocyte differentiation, methods for producing NF-AT proteins for use as res. or diagnostic reagents, methods for producing antibodies reactive with the novel polypeptides, and methods for producing transgenic nonhuman animals.

L15 ANSWER 32 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
1995:541403 Document No. 122:283855 Regulated apoptosis by chimeric proteins binding to FK506-type and cyclosporin-type ligands. **Crabtree, Gerald R.**; Schreiber, Stuart L.; Spencer, David M.; **Wandless, Thomas J.**; Belshaw, Peter (Board of Trustees of the Leland Stanford Junior University, USA; President and Fellows of Harvard College). PCT Int. Appl. WO 9502684 A1 19950126, 134 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, UZ, VN; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US8008 19940718. PRIORITY: US 1993-93499 19930716; US 1994-179143 19940107; WO 1994-US1617 19940214.

AB A general procedure is described for the regulated (inducible) dimerization or oligomerization of intracellular proteins and methods and materials are presented for using that procedure to regulatably initiate cell-specific apoptosis (programmed cell death) in genetically engineered cells. The procedure involves chimeric (or fused) proteins, DNA constructs encoding them, and ligand mols. capable of oligomerizing the chimeric proteins. The chimeric proteins contain at least one ligand-binding (or receptor) domain fused to an action domain capable of initiating apoptosis within a cell, and may also contain addnl. domains for (1) the regulatable or constitutive expression of desired genes and (2) intracellular **targeting.** The chimeric proteins are capable of binding to an FK506-type ligand, a cyclosporin A-type ligand, tetracycline, or a steroid ligand. One such chimeric protein is myristoylated CD3/FKBP12 (MZF3E) receptor consisting of (1) a c-src fragment sufficient for myristoylation, (2) the cytoplasmic tail of ζ (a component of the B cell receptor), (3) 3 consecutive domains of the FKBP12 immunophilin, and (4) a flu epitope tag; oligomerization/apoptosis is induced by a dimeric derivative of FK506. Syntheses are reported for the preparation of dimeric and "bumped" (containing steric bulky groups) derivs. of FK506 and cyclosporin A. The overall procedures allows ligand-mediated oligomerization for regulated gene therapy.

L15 ANSWER 33 OF 36 CAPLUS COPYRIGHT 2005 ACS on STN
1995:377088 Document No. 122:153359 Regulated transcription of target genes with dimeric ligands which cause chimeric receptor proteins to oligomerize and induce gene transcription. **Crabtree, Gerald R.**; Schreiber, Stuart L.; Spencer, David M.; **Wandless, Thomas J.**; Belshaw, Peter (Leland Stanford Junior University, USA; Harvard College). PCT Int. Appl. WO 9418317 A1 19940818, 133 pp. DESIGNATED STATES: W: AT, AU, BB, BG, BR, CA, CH, CN, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, PL, RO, RU, SD, SE, SK; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 1994-US1617 19940214. PRIORITY: US 1993-17931 19930212; US 1993-92977 19930716; US 1994-179748 19940107.

AB A general procedure for regulating (inducing) dimerization or oligomerization of chimeric proteins is presented. The chimeric proteins contain a receptor domain and another protein domain capable of initiating a biol. process. The chimeric proteins can be induced to associate by treating the cells or organisms that harbor them with cell-permeable, synthetic ligands. The dimers/oligomers bind to a transcription control element and stimulate transcription of the gene to which it is associated The syntheses of FK-506 dimers are presented. Such dimers were used to induce: (1) the intracellular aggregation of the cytoplasmic tail of the zeta chain of the T cell receptor (TCR)-CD3 complex thereby leading to signaling and transcription of a reporter gene, (2) the homodimerization of the cytoplasmic tail of the Fas receptor thereby leading to cell-specific apoptosis (programmed cell death) and (3) the heterodimerization of a DNA-binding domain (Gal4) and a transcription-activation domain (VP16) thereby leading to direct transcription of a reporter gene.

L15 ANSWER 34 OF 36 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
94070551 EMBASE Document No.: 1994070551. Calcineurin is a key signaling enzyme in T lymphocyte activation and the target of the immunosuppressive drugs cyclosporin A and FK506. Clipstone N.A.; **Crabtree G.R.**. Howard Hughes Medical Institute, Stanford Univ School of Medicine,Stanford, CA 94305, United States. Annals of the New York Academy of Sciences Vol. 696, pp. 20-30 1993.
ISSN: 0077-8923. CODEN: ANYAA
Pub. Country: United States. Language: English.
ED Entered STN: 940325
DATA NOT AVAILABLE FOR THIS ACCESSION NUMBER

L15 ANSWER 35 OF 36 EMBASE COPYRIGHT 2005 ELSEVIER INC. ALL RIGHTS RESERVED. on STN
92199869 EMBASE Document No.: 1992199869. Inhibition of T cell signaling by immunophilin-ligand complexes correlates with loss of calcineurin phosphatase activity. Liu J.; Albers M.W.; **Wandless T.J.**; Luan S.; Alberg D.G.; Belshaw P.J.; Cohen P.; MacKintosh C.; Klee C.B.; Schreiber S.L.. Department of Chemistry, Harvard University,Cambridge, MA 02138, United States. Biochemistry Vol. 31, No. 16, pp. 3896-3901 1992.
ISSN: 0006-2960. CODEN: BICHAW
Pub. Country: United States. Language: English. Summary Language: English.
ED Entered STN: 920802

AB Calcineurin, a Ca2+, calmodulin-dependent protein phosphatase, was recently found to bind with high affinity to two different immunosuppressant binding proteins (immunophilins) with absolute dependence on the presence of the immunosuppressants FK506 or cyclosporin A (CsA) [Liu et al. (1991) Cell 66, 807-815]. The binding affinities of the immunophilin-drug complexes toward calcineurin and the stoichiometry of the resultant multimeric complexes have now been determined, and structural elements of FK506, CsA, and calcineurin that are critical for mediating their interactions have been identified. Analogues of FK506

(FK520, FK523, 15-O-demethyl-FK520) and CsA (MeBm2t1-CsA and MeAla6-CsA) whose affinities for their cognate immunophilins do not correlate with their immunosuppressive activities have been prepared and evaluated in biochemical and cellular assays. We demonstrate a strong correlation between the ability of these analogues, when bound to their immunophilins, to inhibit the phosphatase activity of calcineurin and their ability to inhibit transcriptional activation by NF-AT, a T cell specific transcription factor that regulates IL-2 gene synthesis in human T cells. In addition, FKBP-FK506 and CyP-CsA do not inhibit members of the PP1, PP2A, and PP2C classes of serine/threonine phosphatases. These data suggest that calcineurin is the relevant cellular target of these immunosuppressive agents and is involved in Ca2+-dependent signal transduction pathways in, among others, T cells and mast cells.

L15 ANSWER 36 OF 36 MEDLINE on STN DUPLICATE 6

90368794. PubMed ID: 2394747. Cell type specificity and activation requirements for NFAT-1 (nuclear factor of activated T-cells) transcriptional activity determined by a new method using transgenic mice to assay transcriptional activity of an individual nuclear factor. Verweij C L; Guidos C; **Crabtree G R.** (Howard Hughes Medical Institute, Stanford, California.) Journal of biological chemistry, (1990 Sep 15) 265 (26) 15788-95. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Nuclear factor of activated T-cells (NFAT-1) is a transcription factor which is considered to be an important regulator in early T-cell activation. We have developed a system to monitor the transcriptional activity of NFAT-1 at the single cell level in whole animals. The system is based on the use of an oligomerized NFAT-1 binding motif that directs transcription of SV40 T-antigen in transgenic mice. This report represents the first demonstration that a multimerized short binding motif can function appropriately in transgenic mice. NFAT-1 activity had previously been thought to be confined to activated T-lymphocytes upon release of intracellular calcium. By **targeting** NFAT-1-dependent gene expression in transgenic mice we discovered new sites of NFAT-1 activity. Besides in T-lymphocytes NFAT-1 activity could also be induced in T-lymphocyte-depleted spleen cells and purified B-lymphocytes and requires agents that both release intracellular calcium and activate protein kinase C. A difference in the time course of appearance of NFAT-1 activity between T-lymphocytes and non-T-lymphocytes was revealed. Constitutive expression was observed in a small population of cells in the dermis and some mice have developed skin lesions. Interestingly, the tissue pattern of expression of the NFAT-1 activity resembles the expression pattern described for HIV-LTR/tat transgenic mice (Vogel, J., Hinrichs, S. H., Reynolds, R. K., Luciw, P. A., and Jay, G. (1988) Nature 335, 606-611). This similarity in expression and the fact that NFAT-1 has been shown to bind functional sequences in HIV-LTR suggest a role for NFAT-1 in dermal activation of the HIV-LTR.